Chiang Mai J. Sci. 2013; 40(4) : 593-602
http://it.science.cmu.ac.th/ejournal/
Contributed Paper

Selection of Some Native Microalgal Strains for Possibility of Bio - oil Production in Thailand

Krongkan Janta [a], Jeeraporn Pekkoh [a,b], Sudaporn Tongsiri [c], Chayakorn Pumas [a] and Yuwadee Peerapornpisal*[a,b]

[a] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
[b] Science and Technology Research Institute, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
[c] Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai 50210, Thailand.
*Author for correspondence; e-mail: yuwadee.p@cmu.ac.th

Received: 26 December 2011
Accepted: 9 November 2012

ABSTRACT

The increasing demand for oil by the world's population has resulted in higher global petroleum prices. Many countries have been trying to explore new energy sources. One such example of this would be biodiesel, which is a form of alternative energy derived from animal and plant lipids. Algae, especially microalgae are organisms, which accumulate high fatty acid in their cells. This research was aimed at comparing the growth rate and high lipid content of native microalgal strains (*Chlorella* sp. AARL G008, *Scenedesmus* sp. AARL G022, *Monoraphidium* sp. AARL G044, *Carteria* sp. AARL G045 and *Carteria* sp. AARL G046) with foreign strain (*Nannochloropsis limnetica* SAG 18.99). All cultures of microalgae were cultivated under Thai climate conditions in Jaworski's Medium (JM). It was found that the native strains grew better than the foreign strain. *Scenedesmus* sp. AARL G022, *Monoraphidium* sp. AARL G044 and *Carteria* sp. AARL G045 were found to be the top three strains in terms of growth rate and lipid content. *Carteria* sp. AARL G045, the best promising strain, was selected and cultivated in 2 media: JM and Algal Media (AM). *Carteria* sp. AARL G045 could grow in AM as well as in JM and accumulated similar amounts of total lipids (29.59 $mg.L^{-1}$ and 27.18 $mg.L^{-1}$, respectively). However, AM is ten percent less expensive than JM.

Keywords: alternative energy, *Carteria* sp., lipid content, microalgal cultivation, soxhlet extraction

1. INTRODUCTION

Presently, petroleum is a very important energy source in the daily lives of all people around the world. Therefore, the rapidly increasing price of oil has had a significant effect on consumers worldwide. Many researchers have tried to explore new forms of an alternative energy, particularly gasohol and biodiesel from plant origins. Biofuel is an alternative form of energy, which is degradable and considered to be less toxic [1]. Algae, especially microalgae, are organisms, which have high potential to be used as raw materials for biodiesel, because of their higher potential photosynthetic efficiency, higher levels of biomass production and faster growth compared to other energy crops.

Moreover, fatty acids in algal cells are similar to those found in higher plants which are suitable for biodiesel transformation [2]. Fatty acids in algal cells are similar to those found in higher plants. Some species have up to a 50% lipid content per dry weight, such as *Botryococcus braunii* (25 - 75 % hydrocarbon per g dry weight) [3], *Chlorella* sp. (28-57.9 % lipid content per g dry weigh) [4], *Nannochloropsis* sp. (31 - 68 % lipid content per dry weight) [5]. Therefore, many countries have realized the benefit of microalgal cultivation for biofuel production. However, most of the studies were done in temperate zones, where the ambient temperature is typically around 25°C [6; 7]. Thailand lies in the tropical zone. Thus the lipid produced strains and their cultivated conditions produced in the temperate countries may not be suitable for Thailand or other tropical countries. In addition, there have been very few studies conducted on the production of microalgal oil in tropical countries, especially Thailand. This is despite the fact that many commercial microalgal farms have cultivated microalgae for food supplements and animal feed in Thailand [8].

Therefore, it is of considerable interest to investigate some of the native Thailand microalgae, which possess a particularly fast rate of growth and high fatty acid content. This research focused on green microalgae, which have been maintained in the culture collection of the Applied Algal Research Laboratory (AARL), Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. These microalgae were cultivated under the ordinary climate conditions of Thailand without regulating control, as compared with the foreign strain, *Nannochloropsis limnetica* SAG 18.99. Thailand's indigenous strains of algae can be considered quite promising as model strains in the production of biofuel within the country.

2. MATERIAL AND METHODS

2.1 Algae Cultures and Cultivation Media

Chlorella sp. AARL G008, *Scenedesmus* sp. AARL G022, *Monoraphidium* sp. AARL G044, *Carteria* sp. AARL G045 and *Carteria* sp. AARL G046 were provided by the Applied Algal Reseach Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand (Figure 1). These cultures were maintained in Jaworski's medium (JM) which contains (mg per liter): $Ca(NO_3).4H_2O$ 20, K_2HPO_4 12.4 , $MgSO_4.7H_2O$ 50 , $NaHCO_3$ 15.9 , EDTA Na_2 2.25 , H_3BO_3 2.48, $MnCl_2.4H_2O$ 1.39, Vitamin B12 0.04, Vitamin B1 0.04, Biotin 0.04, $NaNO_3$ 80 and Na_2HPO_4 14.28 [9]. Algal medium which contains (mg per liter): K_2HPO_4 250, $MgSO_4.7H_2O$ 513, $NaNO_3$ 1000, $NHCl_4$ 50, $CaCl_2$ 58 and $FeCl_3.6H_2O$ 3 as described by Stein (1973) was also used.

The foreign reference strain *N. limnetica* SAG 18.99 was obtained from Culture Collection of Algae (SAG), University of Göttingen, Göttingen, Germany. The algal was grown in basal medium which contains (mg per liter): KNO_3 200, K_2HPO_4 20, $MgSO_4.7H_2O$ 20, soil extract 20, $ZnSO_4.7H_2O$ 5, $MnSO_4.4H_2O$ 5, H_3BO_3 10, $Co(NO_3)_2.6H_2O$ 1, $Na_2MnSO_4.2H_2O$ 1, $CuSO_4.5H_2O$ 0.025 and $FeSO_4.7H_2O$ 35 as described by Schlösser [9].

The seed cultures were cultured at 25°C under continuous shaking at 120 rpm and continuous 10.8 $\mu mol.m^{-2}.s^{-1}$ illumination by florescent lamp in 1000 mL of JM for the native strains and basal medium for *N. limnetica* SAG 18.99.

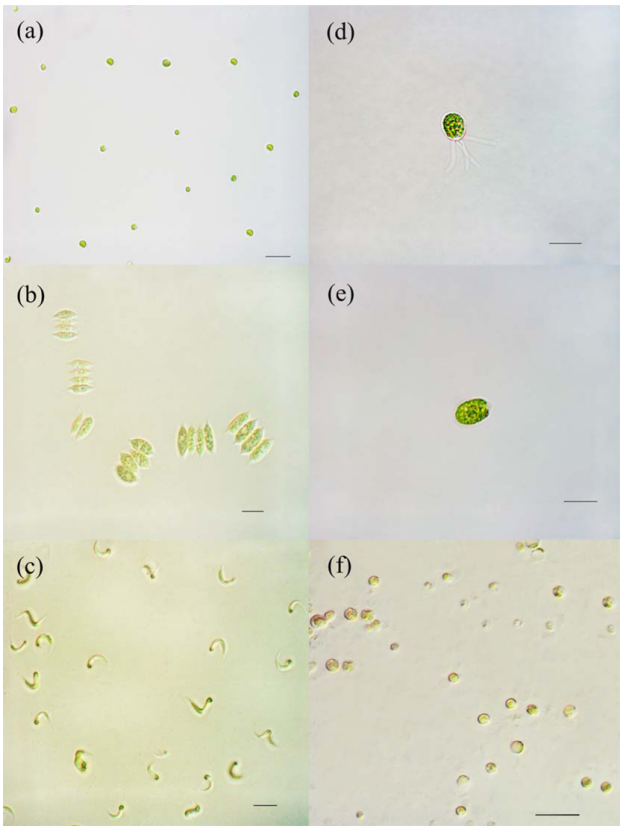

Figure 1 The shape of all microalgae under compound microscope (a) *Chlorella* sp. AARL G008 (b) *Scenedesmus* sp. AARL G022 (c) *Monoraphidium* sp. AARLG044 (d) *Carteria* sp. AARL G045 (e) *Carteria* sp. AARL G046 (f) *Nannochloropsis limnetica* SAG 18.99. Scale bar = 10 μm.

2.2 Growth of Native Thai Strains Compared with Foreign Strain (*N. limnetica* SAG 18.99)

Ten percent (V/V) of each stock culture was inoculated to 10 L of medium cultivated at room temperature with continuous illumination and aeration by bubbling air-line in plastic carboy tank. The growth of each strain was spectrophotometerically measured at OD_{665} nm and cell numbers were counted under light compound microscope by haemacytometer counting chamber every two days. When each culture reached the stationary phase, cells were harvested by centrifugation and dried at 60°C for 48 hr, lipid extraction was prepared by pulverization of dried cell in mortar and extracted by hexane using soxhlet extraction [11]. The growth and lipid accumulation of the native strains were compared with the foreign strain.

2.3 Growth in Low Cost Medium

The stock culture of *Carteria* sp. AARLG045 was washed with sterilized distilled water before being transferred to algal medium (AM) and Jaworski's medium (JM) to obtain the initial optical density at 665 nm (OD_{665}) approximately 0.05. The cultures were cultivated at 25°C under continuous shaking at 120 rpm and continuous 10.8 $\mu mol.m^{-2}.s^{-1}$ illumination by florescent light. When the culture reached stationary phase, biomass and lipid accumulation were analyzed as described above.

2.4 Analytical Method

2.4.1. Growth analysis

The growth rate could be determined by specific growth rate following the equation below.

$$\text{Specific growth rate}(\mu) = \ln(N2/N1)/(t2-t1) \quad (1)$$

Where N_1 and N_2 are the numbers of cells at time 1 (t_1) and time 2 (t_2), respectively [12]

The divisions per day and the doubling time can also be calculated once the specific growth rate is obtained from the following equations (2) and (3), respectively.

$$\text{Divisions per day (Div.day}^{-1}) = \mu/\ln 2 \quad (2)$$

$$\text{Doubling time} = 1 / \text{Div.day}^{-1} \quad (3)$$

2.4.2. Statistical analysis

The data were expressed as mean ± S.D. Statistical comparison between groups was analyzed using SPSS for Windows™ version 14.0.

3. RESULTS AND DISCUSSION

3.1 Growth of Native Thai Strains Compared with Foreign Strain (*N. limnetica* SAG 18.99)

The growth rate of each strain was demonstrated in Figure 2. The highest cell concentration was observed in *Chlorella* sp. AARL G008 (4.6×10^8 cell. mL^{-1}, OD_{665} 0.94, day 20th), followed by *N. limnetica* SAG 18.99 (3.1×10^8 cell.ml^{-1}, OD_{665} 0.72, day 26th), *Monoraphidium* sp. AARL G044 (5 x 10^7 cell. mL^{-1}, OD_{665} 1.69, day 30th), *Scenedesmus* sp. AARL G022 (4.3×10^6 cell.mL^{-1}, OD_{665} 0.95, day 26th), *Carteria* sp. AARL G046 (0.8×10^6 cell.mL^{-1}, OD_{665} 0.40, day 16th), and *Carteria* sp. AARL G045 (1.8×10^6 cell.mL^{-1}, OD_{665} 0.57, day 18th), respectively. When the growth reached the stationary phase, the cells were harvested by centrifugation. It was found that the highest biomass was obtained from *Scenedesmus* sp. AARL G022 (0.67 $g.L^{-1}$), while *N. limnetica* SAG 18.99 produced the lowest biomass (0.24 $g.L^{-1}$)(Figure 3).

Average doubling time of the six algae was calculated in the exponential phase (Figure 4). The high growth rate was observed in *Chlorella* sp. AARL G008, *Carteria* sp. AARL G045 and *Monoraphidium* sp. AARL G044 and the average doubling time was at 78.03, 88.88 and 124.69 hr in the exponential period between the first day to the 14th day, the 12th day and the 24th day, respectively. The low growth rate strains were *Scenedesmus* sp. AARLG 022, *N. limnetica* SAG 18.99 and *Carteria* sp. AARL G046 with the average doubling time of 163.26, 165.01 and 167.81 hr in the exponential period between the first day to the 18th day, the 26th day, and the 12th day, respectively.

The lipid accumulation in algae biomass was analyzed by hexane-soxhlet extraction. The two high lipid content strains were *Monoraphidium* sp. AARL G044 with 11.23% lipid content per dry weight (41.06 $mg.L^{-1}$) and *Carteria* sp. AARL G045 with 7.87 % lipid content per dry weight (41.88 $mg.L^{-1}$). The lowest lipid accumulation was found in *N. limnetica* SAG 18.99 at 4 % lipid content of dry weight (9.72 $mg.L^{-1}$).

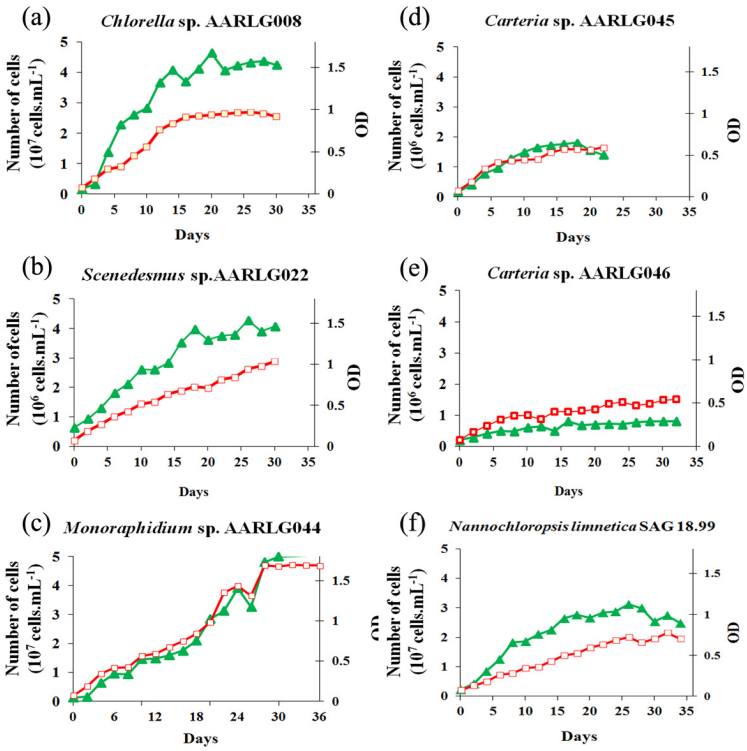

Figure 2 Comparison of growth curves for the different microalgae strains with cell numbers (%) and optical measurement of density; (□). (A) *Chlorella* sp. AARL G008, (B) *Scenedesmus* sp. AARL G022, (C) *Monoraphidium* sp. AARL G044, (D) *Carteria* sp. AARL G045, (E) *Nannochloropsis limnetica* SAG 18.99, (F) *Carteria* sp. AARL G046.

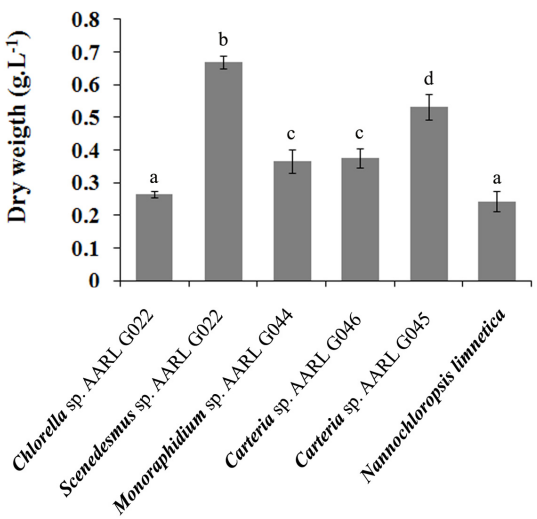

Figure 3 Biomass of microalgae by dry weight measurement ($g.L^{-1}$) which cultivated in Jaworski's medium. Letters on the top (a, b, c and d) are a statistical comparison among groups using analysis of variance (ANOVA) and post-hoc least-significant difference (LSD) test, which significant difference ($p < 0.05$) exists.

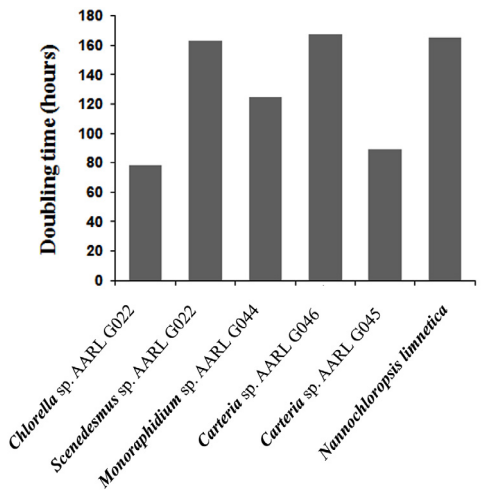

Figure 4 Doubling time of microalgae in the exponential phase.

Many prior research studies have aimed into algal cultivation for the purpose of biodiesel production [5; 13-15]. Nevertheless, these researches were conducted in the temperate zone, where the cultivating temperature was controlled at 25°C. However, Thailand locates in a tropical zone which has a temperature range between 28-33°C. Many researchers have reported that *N. limnetica* SAG 18.99 had a high potential for lipid extraction and therefore could be converted to biodiesel. Chisti (2007) reported that this strain contained high levels of fatty acid (31 - 68% per dry weight) [5]. *N. limnetica* SAG 18.99 showed 25% per dry weight with 1.5 g. $L^{-1}.day^{-1}$ of biomass, when it was cultivated in a pond of 100 L [7]. However, from our results, *N. limnetica* showed the lowest biomass and lipid content under cultivation without temperature control. The study was carried out between June-November, 2010, the lowest temperature was in the range of 25-28.5°C and the highest temperature was between 29.5-32°C. Consequently, this suggested that the foreign well-algal strain may not be suitable for use in Thailand and we therefore, emphasize the need to further explore native algal strains to achieve optimum results in the search for alternative energy sources.

Although, *Chlorella* sp. AARL G008 and *N. limnetica* SAG 18.99 were found to be the two high cell concentrations, the dry weights of these two algae were the lowest due to their small cell size (Figure 5). The production of algae biomass not only depends on the growth rate or cell concentration but also on the size or biovolume of the cell. A barrier to developing algae as a bio-energy source is that it is hard to obtain a high biomass. In addition, small cell size makes problem and increase cost of harvesting biomass [16]. Thus, for the purpose of strain selection, all required properties including growth rate, biomass, cell size and lipid production level should be taken into consideration. *Scenedesmus* sp. AARL G022, *Monoraphidium* sp. AARLG044 and *Carteria* sp. AARLG045 showed potential to be used for further study. Among the three strains, *Scenedesmus* sp. AARL G022 showed the highest amount of biomass but had the lowest lipid content, while the opposite was revealed for *Monoraphidium* sp. AARLG044. *Carteria* sp. AARLG045 was placed in a moderate position. Moreover, *Carteria* sp. AARLG045 reached the stationary phase the fastest and was considered to be relatively

large in terms of cell size. Therefore this strain was chosen for further study. Their lipid contents were lower than in other reports. The green microalgae have lipid content up to 30-50% of their dry weight [5, 17] respectively, *Scenedesmus rubescens*, for which it has been reported that this species could accumulate up to 73% of its dry weight [18]. However, this experiment was only conducted in the initial stage. The optimization of the media and cultivation conditions may improve the growth and lipid production. In addition. *Carteria* sp. AARL G045 was a novel strain for lipid production. The lipid content in the algae biomass in this research was based on only hexane soxhlet extraction, which may not be the best method for fatty acid extraction from *Carteria* sp. AARL G045. Because fatty acid in green microalgae usually contained with short chain hydrocarbon (C 12-22) which should be extracted with chloroform and methanol instead of hexane [2]. Consequently, the comparison of lipid extraction from this strain should be included in the further study.

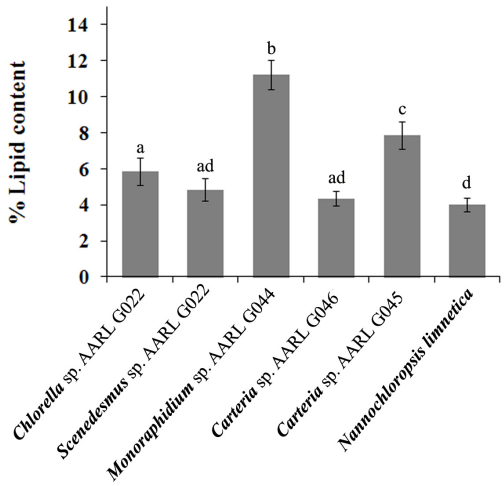

Figure 5 Lipid content of microalgae which cultivated in Jaworski's medium.. Letters on the top (a, b, c and d) are a statistical comparison among groups using analysis of variance (ANOVA) and post-hoc least-significant difference (LSD) test, which significant difference ($p < 0.05$) exists.

3.2 Growth in Low Cost Medium

AM is the basic medium for algal isolation and cultivation [19] which has been widely used. The comparison between cultivation of *Carteria* sp. AARL G045 in JM and AM showed a similar pattern both in terms of the number of cells and the absorbance at 665 nm. The alga reached stationary phase on the 16th day with cell concentration at 1.06×10^6 cell.mL^{-1} and on the 14th day with cell concentration at 1.03×10^6 cell.mL^{-1} when cultivated in AM and JM respectively (Figure 6). The dry biomass samples obtained from AM and JM were not significantly different (p<0.05) as follows: AM (0.42 g.L^{-1}) and JM (0.41 g.L^{-1}) (Figure 7). Although, percentage of lipid content from cultivation in the two media was similar, the total lipid accumulation in AM (7.10 % lipid content of dry weight, 29.59 mg.L^{-1}) was significantly higher than JM (6.6 % lipid content of dry weight, 27.18 mg.L^{-1}) (p<0.05). This may due to the combined effect of having slightly higher biomass and lipid content.

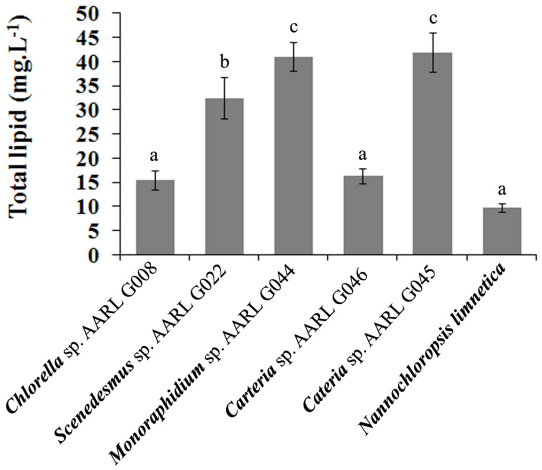

Figure 6 Total lipid content of microalgae in milligrams per liter which cultivated in Jaworski's medium. Letters on the top (a, b, c and d) are a statistical comparison among groups using analysis of variance (ANOVA) and post-hoc least-significant difference (LSD) test, which significant difference ($p < 0.05$) exists.

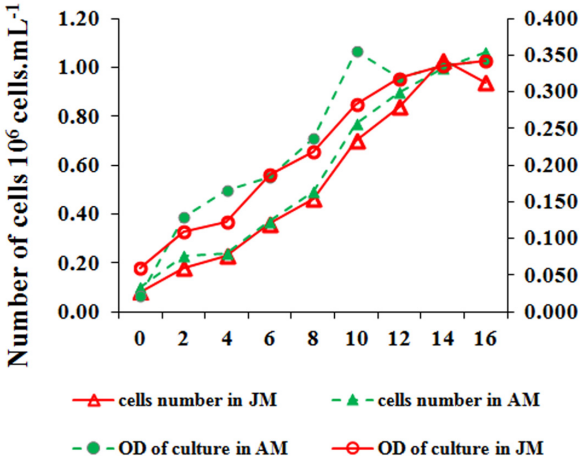

Figure 7 The growth rate and cell number of *Carteria* sp. AARLG045, comparing Jaworski's medium with algal medium.

From the results the cultivation in AM showed the same level of productivity as JM. However, the cultivation in AM was more practical than JM because it contains fewer ingredients. In addition, the cost of AM is lower than JM by about 11% (Table 1). The expensive components in JM are the vitamins, although they are used in small amounts.

Interestingly, AM does not contain any carbon source and trace elements but the nutrient deficiency of AM did not affect the algal growth or lipid production. Thus, it should be of interest as a supply of a cheap carbon source and trace elements in AM can enhance the growth of *Carteria* sp. AARL G045 at a low cost.

Table 1 Comparison of media cultivation cost in Jaworski's medium (JM) with algal medium (AM)

chemicals	Jaworski's medium (JM) prices US$ per unit	Conc. ($g.L^{-1}$)	cost ($US\$.L^{-1}$)	chemicals	Algal medium (AM) prices US$ per unit	Conc. ($g.L^{-1}$)	cost ($US\$.L^{-1}$)
$Ca(NO_3).4H_2O$	12 US$/500g	0.02	0.00048	$NaNO_3$	11.6 US$/500g	1	0.0232
K_2HPO_4	11.2 US$/500g	0.0124	0.00028	$NHCl_4$	9 US$/500g	0.05	0.0009
$MgSO_4.7H_2O$	9 US$ /500g	0.05	0.00090	$MgSO_4.7H_2O$	9 US$/500g	0.513	0.0009
$NaHCO_3$	15.17 US$/1000g	0.0159	0.00024	$CaCl_2$	6.4 US$/500g	0.058	0.00074
EDTA Na_2	37.6 US$/1000g	0.00225	0.00008	$FeCl_3$	18 US$/500g	0.003	0.00011
H_3BO_3	8.8 US$/500g	0.00248	0.00004	K_2HPO_4	11.2 US$/500g	0.25	0.0056
$MnCl_2.4H_2O$	26 US$/500g	0.00139	0.00007				
Vitamin B12	83.33 US$/0.1g	0.00004	0.03333				
Vitamin B1	28 US$/25g	0.00004	0.00004				
Biotin	171 US$ /1g	0.00004	0.00684				
$NaNO_3$	12.8 US$/500g	0.08	0.00248				
Na_2HPO_4	15.2 US$/500g	0.01428	0.00043				
		total	**0.04521**			**total**	**0.03145**

Cost in UsS was transformed from Thai Bath by using 30 Baht = 1 US$

Although AM is cheaper than JM, the long-term cultivation for seed or stock culture, trace elements is necessary. The lack of micro nutrients in AM may not be enough to sustain long term cultivation as was evident in *Spirulina* cultivation. Even though, *Spirulina* grew well in simple media, stock cultures still need some supplemental nutrients in Zarrouk medium [8]. As the culture used in stock culture for seeding should be strong and healthy, it should be maintained in complete media such as JM.

5. CONCLUSION

This study was aimed to select the fast growing and high lipid content Thai microalgal strains compared with the foreign strain (*N. limnetica* SAG 18.99). It was found that the Thai microalgal strains grew better than the foreign stain in tropical climate. Medium cost for the cultivation of microalgae can be reduced by ten percent when microalgae are cultivated in AM with no effect on growth and lipid production. *Carteria* sp. AARL G045, the best promising strain due to high growth rate, high lipid content and cell size.

ACKNOWLEDGEMENTS

The work was supported by The Research Professional Development Project under the Science Achievement Scholarship of Thailand, Office of National Research Council of Thailand. This work was partially financed with support from the National Research Council of Thailand (NRCT) under management of the Institute for Science and Technology Research and Development, Chiang Mai University. We thank Department of Biology, Faculty of Science, Chiang Mai University for their support.

REFERENCES

[1] Petchmala A., Yujaroen D., Shotipruk A., Goto M., Sasaki M., Production Methyl Esters from Palm Fatty Acids in Supercritical Methanol, Chiang Mai J. Sci., 2008; **35:** 23-28.

[2] Amaro H.M., Guedes A.C., Malcata F.X., Advances and Perspectives in Using Microalgae to Produce Biodiesel, Appl. Energy., 2011; **88:** 3402-3410.

[3] Metzger P. and Largeau C., *Botryococcus braunii*: a Rich Source for Hydrocarbons and Related Ether Lipids, Appl. Microbiol. Biot., 2005; **66:** 486-496.

[4] Miao X., and Wu Q., High Yield Bio-oil Production from Fast Pyrolysis by Metabolic Controlling of *Chlorella protothecoides*, J. Biotechnol., 2004; **110:** 85-93.

[5] Chisti Y., Bio-oil from Microalgae, Biotechnol. Adv., 2007; **25:** 294-306.

[6] Attilio C., Alessandro A.C., Erika Y.O., Patrizia P. and Marco D.B., Effect of Temperature and Nitrogen Concentration on the Growth and Lipid Content of *Nannochloropsis oculata* and *Chlorella vulgaris* for Biodiesel Production, Chem. Eng. Process., 2009; **48:** 1146-1151.

[7] Chini Zittelli G., Lavista F., Bastianini A., Rodolfi L., Vincenzini M., Tredici M.R., Production of Eicosapentaenoic Acid by *Nannochloropsis* sp. Cultures in Outdoor Tubular Photobioreactors, J. Biotechnol. 1999; **70:** 299-312.

[8] Peerapornpisal Y., Cultivation of *Spilurina*, Institute of Science and Technology for Research and Development, Chiang Mai University, Chiang Mai, 2003; **76**.

[9] Thompson A.S., Rhodes J.C. and Pettman I., *Culture Collection of Algae and Protozoa:* catalogue of strains, 5ht Edn., Culture Collection of Algae and Protozoa (CCAP), Freshwater Biological Association, Ambleside, Cumbria, UK, 1988.

[10] Schlösser U., Sammlung von Algenkulturen, Ber. Deutsch. Bot. Ges. 1982; **95:** 181-276.

[11] Wangchai N. Lipid Analysis. Aquatic Animal Nutrition, Aquatic Animal Nutrition, Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand, 2009; 206-207.

[12] Levasseur M., Thompson P.A. and Harrison P.J., Physiological acclimation of marine phytoplankton to different nitrogen sources, J. Phycol., 1993; **29:** 587-595.

[13] Berg-Nilsen J., Production of Micro Algea-Based Products, Nordic Innovation Centre, Oslo, Norway, 2006; **29**.

[14] Danielo O., An Algae-Based Fuel, Biofuture, 2005; 255-259.

[15] Sheehan J., Dunahay T., Benemann J. and Roessler P., A Look Back at the U.S. Department of Energy's Aquatic Species Program-Bio-oil from Algae, The National Renewable Energy Laboratory, Colorado, U.S.A., 1998; **239**.

[16] Demirbas A., Use of Algae as Biofuel Sources, Energy Conversion and Management, 2010; **5:** 2738-2749.

[17] Renaud S.M., Parry D.L., Luong-Van T., Microalgae for Use in Tropical Aquaculture I: Gross Chemical and Fatty Acid Composition of Twelve Species of Microalgae from the Northern Territory, Australia. J. Appl. Phycol. 1994; **6:** 337-345.

[18] Matsunaga T., Matsumoto M. Maeda Y., Sugiyama H., Sato R. and Tanaka T., Characterization of Marine Microalga, *Scenedesmus* sp. Strain JPCC GA0024 Toward Biofuel Production. Biotechnol. Lett., 2009; **31:** 1367-1372.

[19] Stein J.R., Handbook of Phycological Methods, Culture Methods and Growth Measurements, Cambridge University Press, Cambridge, 1973; **578**.