Characterization of Local Microalgae from Sabah for Biofuel Production

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ABSTRACT

Microalgae are currently being promoted as an ideal source for biofuel production because of their higher growth rate, higher biomass production and more efficient photosynthetic rate compared to conventional biodiesel sources such as oil palm or soybean. Selection of the right species is the fundamental factor in microalgae-based biodiesel production. Many microalgae species have been isolated and characterized for lipid production; however, there is currently no confirmation as to which species provide the highest productivity. Different species are expected to function best at different aquatic, geographical and climatic conditions. The aims for this study were; (1) to identify local algal species with biofuel potential based on three criteria, namely; lipid content, growth rate and fatty acids methyl esters (FAME) profile, (2) to optimize the culture conditions and (3) to extract lipids from the candidate species. A total of eight microalgal species from local marine and freshwater habitats were isolated and identified based on their morphological characters and molecular characterization of the ribosomal 18S region. Of these, four candidate species (Chaetoceros muelleri, Isochrysis galbana, Chlorella emersonii and Ankistrodesmus falcatus) were chosen based on the criteria above for mass cultures. Analyses showed that the lipid content for each candidate species is in a desired range (between 20 – 45 %) and when grown under nitrogen limitation, the cells were composed of C16/C18 fatty acids, which is suitable for biodiesel synthesis. Subsequently, these species will be mass cultured and studies on their ability to produce biofuel will be carried out.

Keywords: algae, biofuel, lipid, fatty acids

INTRODUCTION

Microalgae is classified as the third generation biofuel feedstock, following lignocellulosic biomass as the second generation and the first generation
feedstock which are sugars, grains and seeds (Feng et al., 2012). It has been widely recognized as a promising potential feedstock of biofuel due to its higher growth rate, higher biomass production, unnecessary requirement for arable land and more efficient photosynthetic rate compared to conventional biodiesel sources such as oil palm or soy bean. Species with high oil yields can be extracted, processed and enhanced into transportation fuels (Gouevia et al., 2009).

The success of microalgae-based biodiesel production as feedstock depends strongly on the selection of candidate algal species (Zhou et al., 2013). Selection of right candidate species with characteristics such as fast-growing, productive strain and optimized for the local climatic conditions has significant importance as it marks the beginning of any algal mass cultivation. Many microalgal species have been isolated and characterized for lipid production; however, for tropical microalgae, there is currently no confirmation as to which species provide the highest oil productivity. Different species are expected to function best at different aquatic, geographical and climatic conditions. A thorough selection and optimization of any algae species are needed in order to achieve a satisfactory lipid yield and thus, improving the production and performance of the obtained biofuel (Mutanda et al., 2011; Duong et al., 2012).

Up to now in Sabah, there has been no research on the potential of local microalgae species to produce biofuel based on fatty acids composition associated with lipid production. This research will lead to the identification of local microalgae capable of producing large quantities of lipids as a potential candidate for biofuel production which eventually will facilitate in providing data for future studies regarding biofuels from local microalgae. At the end of this study, the selected algae species with desired criteria can be utilized for biodiesel commercialization. Thus, creating and opening up avenues for production of biofuel in Sabah and indirectly contribute to the development of algal biotechnology in Sabah.

Microalgae are a promising alternative source of oil for biodiesel production. Identification of a species with desirable characteristics is a key component towards achieving economic feasibility for the process. This has been compromised by the lack of data allowing effective interspecies comparison. The feasibility of algae-based biodiesel industry depends on the selection of appropriate strains which leads to profitable yields and oil quality. Additionally, cultivation of algal species for biofuel production is well-documented in other temperate countries, but there seems to be insufficient information on the potential of tropical microalgal species for biofuel production. Thus, screening
of local microalgal species with biofuel potential can contribute in selecting potential candidate species.

**MATERIALS AND METHODS**

**Sample collection and isolation of microalgae**

Microalgal samples were collected from freshwater and marine habitats such as lakes, ponds and sea in Kota Kinabalu area. Plankton net with a mesh size of 20 microns (µ) was used during the sampling. Isolation was done by micropipette washing technique (Phang and Chu, 1999). Media that were used were the BBM (Bold’s Basal Medium) and Walne’s medium. In this technique, a very fine-tipped pipette was used to capture single cells from the collected sample under inverted microscope. The fine-tipped pipette can be made by holding a glass Pasteur pipette in a flame of a Bunsen burner until it become slightly soft. Then the pipette was moved from the flame and stretched out using forceps to create a very thin but hollow glass tube. This fine-tipped pipette was sterilized and plugged with cotton before it is ready for use.

Ten drops of BBM media were placed on a glass slide. Then a drop of the collected sample was added to the first drop and observed under an inverted microscope. After confirming the target species, a drop of the sample was transferred to the next one. This step was repeated for 6 to 7 times to obtain a unialgal culture as well as to minimize contamination. After washing it for several times, a final drop of the sample which contained only one alga was transferred into a well of the culture plate. One ml of the BBM media was dropped inside one of the well before transferring the isolated algae into it. The cells were then observed daily and when the cells have multiplied, it was transferred into a 100 ml flask for culture purpose (Phang and Chu, 1999).

**Molecular identification of isolated species**

Genomic DNA was extracted by using Nucleospin® 96 Plant II kit (Macherey-Nagel, Germany). About 100 to 150 ml of algal culture was centrifuged in Falcon tubes at approximately 16,000 x g for 1 minute. The supernatant was discarded and the pellet was subjected to DNA extraction following the manufacturer’s instructions. The 18S rDNA region was amplified using universal primers 18ScomF1 (forward), 5’-GCTTGTCTCAAAGATTAAGCCATGC-3’, and 18ScomR1 (reverse), 5’ CACCTACGGAAACCTTGTTACGAC-3’) (Zhang et al., 2004). The total PCR reaction volume was 50 µl, containing 1.25 U of TopTaq DNA Polymerase (QIAGEN, Germany), 1 X of TopTaq PCR Buffer® (QIAGEN, Germany), 1 X of Q-Solution (QIAGEN, Germany), 200 µM of each dNTPs (Fermentas, Lithuania), 1 pmol of each primer and 50 ng template DNA. PCR amplifications were
performed with an initial denaturation step of 94°C for 3 min followed by 5 cycles of denaturation at 93°C for 1 min, annealing at 45°C for 1 min and extension at 72°C for 1 min, and additional of 30 cycles of denaturation at 93°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 30 sec, and a final extension step at 72°C for 5 min. The PCR products were purified by using the QIAquick Gel Extraction Kit (QIAGEN, Germany) in accordance with the manufacturer’s protocol before the purified PCR products were cloned into a pJET1.2 blunt cloning vector (Fermentas, Lithuania).

RESULTS AND DISCUSSION

According to the obtained 18S rDNA sequences, it can be concluded that microalgal isolates FW1, FW2, FW3, FW4, SW1, SW2, SW3 and SW4 were closely related to *Ankistrodesmus fusiformis*, *Chlamydomonas monadina*, *Chlorella emersonii*, *Scenedesmus obliquus*, *Chaetoceros muelleri*, *Isochrysis galbana*, *Nannochloropsis oculata* and *Tetraselmis chuii* with 93%, 96%, 98%, 98%, 97%, 98%, 98% and 97% sequence similarities, respectively. All the 18S rDNA sequences of both marine and freshwater isolates used were deposited into GenBank nucleotide sequences database with the accession numbers: KC594685 – KC594688 and KC852902 – KC852905. Table 1 shows the microscopic images of the eight species isolated from freshwater and marine habitat in Kota Kinabalu and it was observed that there were several dissimilar features among the isolates.

Microalgae identification is a major challenge encountered by researchers around the world (Ratha et al., 2011). The term morphology is referring to the shape, form or growth habit of an organism and its part. Algae exhibit incredibly diverse morphology where the estimated number of species are approximately 200,000 to 800,000 (Ebenezer et al., 2011). There are non-motile and motile, flagellated and non-flagellated, unicellulars and colonies.

Conventional approach such as morphological characterization has been acknowledged as important tools in defining the algal flora of a particular habitat. Furthermore, there are some researchers that still hinge on this technique (Flechtner et al., 1998, 2008; Škaloud, 2009). Morphological characterization is commonly carried out using light microscope. However, there are limitations to this technique. Many algae are required to be observed from its cultured media in order to observe motile stages. Species such as *Chlorella*, which has a small size and simple morphological structure, does not produce alternative life stages such as gametes and zoospores. Trainor and Egan (1990) and Sluiman and Gärtner (1990) proved in their studies that nutrient composition or physical
form of a substrate medium can cause morphological plasticity for members of genera *Scenedesmus* and *Pleurastrum*, respectively. Additionally, environmental factors can also influence algal morphology (Luo *et al.*, 2006). The presence of plasticity can cause the classification of an isolate in the correct taxonomic group using morphological characteristics to be difficult. Thus, it is crucial to provide morphological data that are acquired through light microscopy with molecular analysis or ultrastructural characteristics (Flechtner *et al.*, 2013).

**ACKNOWLEDGEMENT**
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**REFERENCES**
### Table 1 Microscopic images of eight microalgae isolated from freshwater and marine habitat in Kota Kinabalu.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>MICROSCOPE IMAGES</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW1</td>
<td><img src="image1" alt="Image" /></td>
<td>Species: Ankistrodesmus sp.&lt;br&gt;Division: Chlorophyta&lt;br&gt;Media: Bold's Basal Media (BBM)&lt;br&gt;Location sampled: Tun Fuad Stephens</td>
</tr>
<tr>
<td>FW2</td>
<td><img src="image2" alt="Image" /></td>
<td>Species: Chlamydomonas sp.&lt;br&gt;Division: Chlorophyta&lt;br&gt;Media: Bold's Basal Media (BBM)&lt;br&gt;Location sampled: Tun Fuad Stephens</td>
</tr>
<tr>
<td>FW3</td>
<td><img src="image3" alt="Image" /></td>
<td>Species: Chlorella sp.&lt;br&gt;Division: Chlorophyta&lt;br&gt;Media: Bold's Basal Media (BBM)&lt;br&gt;Location sampled: UMS Hatchery</td>
</tr>
<tr>
<td>FW4</td>
<td><img src="image4" alt="Image" /></td>
<td>Species: Scenedesmus sp.&lt;br&gt;Division: Chlorophyta&lt;br&gt;Media: Bold's Basal Media (BBM)&lt;br&gt;Location sampled: UMS Hatchery</td>
</tr>
<tr>
<td>SW1</td>
<td><img src="image5" alt="Image" /></td>
<td>Species: Chaetoceros sp.&lt;br&gt;Division: Heterokontophyta&lt;br&gt;Media: Wahe's Media (WM)&lt;br&gt;Location sampled: KK Wetland</td>
</tr>
<tr>
<td>SW2</td>
<td><img src="image6" alt="Image" /></td>
<td>Species: Isochrysis sp.&lt;br&gt;Division: Haptophyta&lt;br&gt;Media: Wahe's Media (WM)&lt;br&gt;Location sampled: Sepanggar Bay</td>
</tr>
<tr>
<td>SW3</td>
<td><img src="image7" alt="Image" /></td>
<td>Species: Tetraselmis sp.&lt;br&gt;Division: Chlorophyta&lt;br&gt;Media: Wahe's Media (WM)&lt;br&gt;Location sampled: BMRI Fish Tank</td>
</tr>
<tr>
<td>SW4</td>
<td><img src="image8" alt="Image" /></td>
<td>Species: Nanochloropsis sp.&lt;br&gt;Division: Heterokontophyta&lt;br&gt;Media: Wahe's Media (WM)&lt;br&gt;Location sampled: BMRI Fish Tank</td>
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