LIPID PRODUCING MICROALGAE FROM SEVERAL ECOSYSTEMS IN WEST AND CENTRAL JAVA, INDONESIA

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ABSTRACT

This study is aimed to get lipid producing microalgae as feedstock for biofuel production. The microalgae were isolated from 355 collected water samples which represented many distinct ecosystems such as paddy fields, rivers, agricultural dams, ponds, swampy areas and unique ecosystem of volcano and mud-volcano craters in West- and Central Java, Indonesia. A total of 267 strains of microalgae were isolated from the samples of which 221 strains of them have capability to produce lipid. There were four promising strains that produce lipid between 14.7 - 45.7 percent dry weight in optimal condition that were identified as *Chlamydomonas* sp. KO-7267 and PK-7195, *Chlorella* sp. KS-7300 and *Desmodesmus* sp. BK-7291.

Keywords: Microalgae, lipid, biofuel, Indonesian ecosystems

INTRODUCTION

Global oil production is rapidly approaching its peak, even if natural gas liquids and expensive, destructive, and risky deepwater and polar oil are included. Based on several scenarios, the peak oil will happen sometime between 2010 - 2030 (Robert 2005). Peak oil means that half of oil reserves has been exploited and used. Peak production of oil in Indonesia has occurred in the 1990 (Full Report Workbook 2004). In 2004, Indonesia (formerly OPEC member) became a net importer. The lack of stability of future energy supplies has motivated the development of alternative energy sources in order to eliminate the possibility of a future energy shortage. Furthermore, one of the most environmental problems today is global warming, caused primarily due to the heavy use of fossil fuel. In the world, large amount of CO_2

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are released into the atmosphere. Photosynthetic microalgae are potential candidate for substitution of the fossil energy. Some strains can produce lipid which can be converted into biodiesel, while others produce petroleum like hydrocarbon. The cultivation of algae is also important to reduce the effect of climate change through utilizing excessive amount of CO_2, since cultivated these organisms are capable of fixing CO_2 to produce energy and chemical compounds upon exposure to sunlight. Microalgae are photosynthetic renewable resources with high lipid content, have faster growth rate than plant cells and are capable to grow in saline waters which are unsuitable for agriculture. The lipid content of microalgae on a dry cellular weight basis varies between 20 and 40%. Lipid content as high as 85% has been reported for certain microalgal strains. *Botryococcus braunii*, is a unique microalgal strain, having a long hydrocarbon chain of between 30 and 40% (dry weight basis) which produces almost similar compounds as crude oil. Both physical and chemical processes are applicable in the production of liquid fuels from algal strain of high lipid content. These processes include direct lipid extraction in the production of diesel oils substitutes, transesterification in the formation of ester fuels, and hydrogenation in the production of hydrocarbons (Borowitzka 1988).

Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. Algal fatty acids and oils have a range of potential applications. The characteristics of algal oils are similar to those of fish and vegetable oils, and can thus be considered as potential substitutes for the products of fossil oil (as a biodiesel) (Miyamoto 1997).

Cultivation of microalgae in a large scale can be considered as potential substitutes for the petroleum-based diesel used in transportation. The study conducted by NERL (*National Renewable Energy Laboratory*, US) found that 7.5 billion gallon of biodiesel from microalgae could be produced on 200,000 ha of desert land per year. This value equals to 214 million barrels or equivalent to 65% of total annual petroleum production in Indonesia. This value could never be reached by cultivating other energy producing plants.

Indonesian microalgae as sources of biofuel production are almost unexplored. Very few related reports and publications could be found in Indonesia. The exploration of Indonesian microalgae as a source of sustainable energy is therefore very promising.

MATERIALS AND METHODS

Samples collection

Samples were collected from rivers, lakes, paddy fields, ponds, dams, craters and saline waters in the south area of West and Central Java, and also some areas in the northern coast of coastal area of Central Java (Fig. 1). Those areas represent a large variety in soils, pH, temperature and salinity. A number of 355 samples were collected from 36 different sites (Table 1).

Figure 1. The sampling sites

Isolation and screening of microalgae

The soil and fresh water samples were cultured in 616 medium (ATCC) and MJ Medium (Modified Jorgensen's media for diatoms). The samples from the saline habitats were cultured in modified NORO medium (Takagi *et al.* 2005) containing (per litre): NaCl 29.2g; KNO_3 1g; $MgCl_2.H_2O$ 1.5g; $MgSO_4.7H_2O$ 0.5g; KCl 0.2g; $CaCl_2$ 0.2g; K_2HPO_4 0.045g; tris(hydroxymethyl)aminomethane 2.45g; EDTA.2Na 1.8g; ZnSO47H2O 0.087 mg;H_3BO_3 0.61 mg; $CoCl_2.6H_2O$ 0.015mg; $CuSO_4.5H_2O$ 0.06 mg; $MnCl_2$ 0.23mg; $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.38mg; Fe(III)EDTA 3.64 mg. The pH was adjusted to 8.0 with 1N HCl. Other culture media were used in order to obtain suitable media for their growth. The algae were subjected to purification by serial dilution followed by plating. The individual colonies were isolated and inoculated into liquid medium and incubated at 27±2 °C under 1.2±0.5 klux light intensity with 12:12 hrs photoperiods. The purity of culture was ensured by repeated plating and by regular observation under microscope.

The isolates were characterized with respect to lipid and/or oil production capabilities. The lipid and oil producing microalgae were determined by using nile red (NR) staining (Qin 2005). A stock solution of NR was prepared by adding 2.5 mg of NR to 100ml of acetone. The solution was kept in an amber-coloured bottle and stored in the dark at room temperature. The microalgae cell was stained by placing 5 ml of culture in a Petridish, then added with 200µl of NR stock solution to the dish. Staining of lipids and oil was completed within 30 minutes. No destaining or rinsing was required. After staining, a drop of stained cell culture was transferred to a depression slide and cover slip added. Cell culture was examined at 1000x magnification using a phase contrast microscope and epiflourescence microscope. When viewed under epiflourescent illumination, neutral lipid and oil of microalgae stained with NR will show a bright yellow/orange color (Carman1991; Qin 2005).

The growth of microalgal culture was determined daily based on the appearance of green or brown color on the medium. The cell density was measured with a spectrophotometer using 680 nm wavelengths (Lee *et al.* 1998). The terminal optical density (OD) will mark at 0.2, at this point the alga biomass is equivalent to 0.37g of dry weight per liter (Qin 2005). The isolates were screened based on the period of time to reach OD 0.2. The total lipid production of the selected strains was measured by gravimetry methods.

RESULTS AND DISCUSSIONS

Sampling

Sample collection was conducted in West and Central Java from April 10 until April 14, 2008. Samples were taken from diverse ecosystems i.e. sampling in mountain ecosystem in West Java (Gunung Gede, Gunung Tangkuban Perahu), volcanic lakes (crater of Tangkuban Perahu, pre-historic crater of Bledug Kuwu), rivers, lakes, ponds, and rice fields in West and Central Java, as well as swampy areas in northern part of Central Java. Sampling has also been conducted in two agricultural dams i.e. Waduk Sempor, Kebumen and Waduk Kedung Ombo, Purwodadi. A number of 355 water samples have been collected from those areas. The samples and sampling location are shown in Table 1.

Table 1. Samples and sampling locations

Code of Samples	Sampling locations	Characteristic
CP7041-CP7050	Ciputri, Pacet, Cianjur, West Java	Water from paddy fields and ponds
SK7051-SK7055	Sukamantri, Karangtengah, West Java	Water from paddy fields
CB7056-CB7060	Ciburung, Padalarang, West Java	Water from dam
TP7061-TP7080	Tangkuban Perahu Mountain, West Java	Water from crater, hot-sulfuric water
JL7081-JL7085	Jaya Giri, Lembang, West Java	Water from fishponds
PT7086-PT7090	Highway Kopo, West Java	Water from paddy fields
CG7091-CG7095	Limbangan street, Garut, West Java	Water from paddy fields
CT7095-CT7100	Ciawi, Tasik, West Java	Water from paddy fields
BE7101-BE7110	Bojongsari, Cijenjing, Ciamis, West Java	Water from fishponds
MH7111-MH7120	Kota Banjar, West Java	Water from river
PD7121-PD7130	Panulisan, Dayeuh Harja, Cilacap, Central Java	Water from paddy fields
CR7131-CR7140	Ciraja, Kt Pucung, Cilacap, Central Java	Water from paddy fields
JL7141-JL7145	Jatilawang, Banyumas, Central Java	Water from paddy fields
KJ7146-KJ7155	Kr.anyar, Jatilawang, Banyumas, Central Java	Water from paddy fields
TB7156-TB7165	Purwodadi, Tambak, Banyumas, Central Java	Water from paddy fields
SMP7166-SMP7190	Waduk Sempor, Kebumen, Central Java	Water from agricultural dam
PK7191-PK7195	Pekunden, Kota Winangun, Kebumen, Central Java	Water from paddy fields
PR7196-PR7200	Purworejo, DIY	Water from paddy fields
TPK7201-TPK7205	Taji, Prambanan, DIY	Water from paddy fields
MC7210-MC7225	Metese, Ceper, Klaten, DIY	Water from paddy fields

Table 1. Continued

Code of Samples	Sampling locations	Characteristic
PW7226-PW7235	Pakis, Wadung Getas, Delanggu, Klaten, DIY	Water from paddy fields
SG7236-SG7245	Siwali, Gondangrejo, Central Java	Water from paddy fields
LS7246-LS7255	Ngadul, Sumbu Lawang, Sragen, Central Java	Water from paddy fields
KO7256-KO7275	Waduk Kedung Ombo, Purwodadi, Central Java	Water from agricultural dam
MT7276-MT7280	Mayahan, Tawangharjo, Purwodadi, Central Java	Water from paddy fields
BK7281-BK7295	Bledug Kuwu, Grobogan, Central Java	Water from crater
KS7296-KS7300	Kuwu, Grobogan, Central Java	Water from paddy fields
TB7301-TB7310	Tambaksari, Blora, Central Java	Water from paddy fields
KSR7311-KSR7315	Kemadu, Sulang, Rembang, Central Java	Water from paddy fields
BKR7316-BKR7340	Banyudono, Kaliori, Rembang, Central Java	Water from fishponds, salt producing ponds
KPR7341-KPR7350	Karangpandan, Rembang, Central Java	Water from fishponds
GR7531-GR7360	Growong, Juwana, Pati, Central Java	Water from paddy fields
TN7361-TN7370	Teban, Njekulo, Kudus, Central Java	Water from paddy fields
TKJ7371-TKJ7380	Tanjang, Karangjati, Central Java	Water from paddy fields
YW7386-YW7390	Jogoloyo, Wono Salam, Demak	Water from paddy fields
KD7391-KD7395	Pangan, Kandanghaur, Indramayu	Water from paddy fields

Isolation and screening of Algae

Microalgae were isolated from samples collected from rivers, lakes, brackish and freshwater ponds, paddy fields, wetland soils in West and Central Java. The method and suitable medium for the isolation of microalgae were developed during this research. The major source of microalgae isolation (155 samples) came from paddy fields. A total of 355 samples have been used for algae isolation and 267 isolates of microalgae were obtained. Some samples produced colony of algae quite fast and the medium became green after 2-3 weeks, while other samples grew slowly or did not grow. Two medium have been used i.e. Modified Jorgensen's medium and ATCC 617 medium. There were no differences between the two medium in supporting the growth of algae. The algal growth depended on the origin sample. The isolates obtained from this research were deposited at Laboratory of Soil Biotechnology of IPB and the duplicates were deposited at ICBB-Culture Collection of Micoorganisms.

Distribution of microalgae based on the Origin samples

It was shown that origin samples play important role on the success of algae isolation. Samples can be grouped into 5 : 1) paddy field, 2) river, 3) ponds, 4) agricultural dam, and 5) crater. Most samples were collected from the paddy fields. The percentage of samples from paddy fields producing colony of algae is shown in Table 2.

Table 2. The percentage and distribution of samples from paddy field that produced algal growth

Sampling site	%	Sampling site	%	Sampling site	%
West Java			Central Java		
Cianjur	70	Cilacap	85	Purwodadi	80
Karangtengah	60	Banyumas	65	Grobogan	80
Bandung	80	Kebumen	45	Blora	90
Garut	80	Purworejo	70	Rembang	80
Tasik	83	Prambanan	80	Pati	63
Indramayu	60	Klaten	70	Kudus	100
		Karanganyar	100	Karangjati	70
		Sragen	80	Demak	90

The geographical locations of sampling showed no significant difference in algal growth. There were also no differences between samples collected from West Java and Central Java. Samples collected from four sites produced microalgae in the range of 90 - 100 percent i.e. Karanganyar, Blora, Kudus and Demak.

Microalgae are abundant and can be found not only in paddy fields but also in all of this project studied ecosystems, both man-made ecosystems such as fish ponds and agricultural dams as well as natural ecosystems. Interestingly, microalgae can also be isolated from harsh environment such as Tangkuban Perahu crater with very low pH (1-2) as well as Bledug Kuwu, a mud volcano crater that contains high concentration of salt. More than 60 percent of the samples, except samples collected from Padalarang Dam, produced microalgae after incubated in an appropriate media (Table 3).

Table 3. Percent growth of microalgae from different ecosystems

Sampling sites	%	Sampling sites	%
Volcano crater, Tangkuban perahu	68	Salt producing ponds, Rembang	87
Mud-volcano crater, Bledug Kuwu	79	River, Banjar	67
Dam, Padalarang	20	Fish pond, Lembang	80
Dam, Sempor	64	Fish pond, Ciamis	60
Dam, Kedung Ombo	78	Fish pond, Rembang	90

Lipid producing microalgae

Around 267 strains of algae could be isolated from the samples. Screening on lipid production of microalgae cell showed that 79 percent of the strains (212 isolates) have capability to produce lipid. Those strains are then selected for further study in order to obtain the best strain which has fast growth and high lipid production to be developed further as a source of biodiesel. Screening had been conducted on those strains and four promising isolates were selected i.e. PK-7195 produced 25.5 percent lipid, BK-7291 produced 14.7 percent lipid, KO-7267 produced 35.7 percent lipid, and KS-7300 produced 45.7 percent lipid under optimum condition. PK-7195 was isolated from a paddy field sample of Pekunden, Kota Winangun, Kebumen, Central Java; BK-7291 was isolated from a sample of mud-volcano crater of Bledug Kuwu, Grobogan, Central Java; KO-7267 was from Kedung Ombo Dam, Purwodadi, Central Java and KS-7300 from sample of paddy field in Kuwu, Grobogan, Central

Java. Based on the morphological characters, isolate KO-7267 and PK-7195 are identified as *Chlamydomonas* sp., isolates KS-7300 identified as *Chlorella* sp., while BK-7291 is *Desmodesmus* sp.

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