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Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31

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ABSTRACT

The growth and lipid productivity of an isolated microalga *Chlorella vulgaris* ESP-31 were investigated under different media and cultivation conditions, including phototrophic growth ($NaHCO_3$ or CO_2, with light), heterotrophic growth (glucose, without light), photoheterotrophic growth (glucose, with light) and mixotrophic growth (glucose and CO_2, with light). *C. vulgaris* ESP-31 preferred to grow under phototrophic (CO_2), photoheterotrophic and mixotrophic conditions on nitrogen-rich medium (i.e., Basal medium and Modified Bristol's medium), reaching a biomass concentration of 2–5 g/l. The growth on nitrogen-limiting MBL medium resulted in higher lipid accumulation (20–53%) but slower growth rate. Higher lipid content (40–53%) and higher lipid productivity (67–144 mg/l/d) were obtained under mixotrophic cultivation with all the culture media used. The fatty acid composition of the microalgal lipid comprises over 60–68% of saturated fatty acids (i.e., palmitic acid (C16:0), stearic acid (C18:0)) and mono-unsaturated acids (i.e., oleic acid (C18:1)). This lipid composition is suitable for biodiesel production.
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1. Introduction

Microalgal biomass, containing lipids, starch, cellulose, proteins, and so on, is considered a promising feedstock for producing a variety of renewable fuels, such as biodiesel, bioethanol, biohydrogen and methane (Carioca, 2010; Chisti, 2008; Posten and Schaub, 2009). Microalgal lipids have attracted much attention as future raw materials for biodiesel synthesis due to the potential of attaining much higher lipid productivity than is possible with other lipid-based energy crops (Chisti, 2007; Griffiths and Harrison, 2009). Several strategies have been applied to improve microalgae growth and lipid content. These include optimization of the medium compositions (e.g., type of carbon source, vitamins, salts and nutrients), physical parameters (e.g., pH, temperature and light intensity), and type of metabolism (e.g., phototrophic, heterotrophic, mixotrophic and photoheterotrophic growth) (Chojnacka and Marquez-Rocha, 2004; Mata et al., 2010). In particular, the type of cultivation method, using different energy sources (light or organic) and carbon sources (inorganic or organic), is always recognized as a key factor that significantly influences the growth and lipid accumulation of microalgae (Chen et al., 2011; Mata et al., 2010).

Photoheterotrophic conditions are rarely mentioned in the literature, and it is usually confusing to distinguish mixotrophic from photoheterotrophic growth (Griffiths and Harrison, 2009; Mata et al., 2010). In photoheterotrophic cultivation, the microalgae require light as an energy source while using organic materials as the carbon source. In contrast, both organic carbon and CO_2 are essential carbon sources in mixotrophic growth, as a light source is also supplied (Chojnacka and Marquez-Rocha, 2004). Although photoautotrophic conditions are commonly used in microalgae cultivation, some reports noted that higher biomass and lipid productivity of *Chlorella* sp. could be obtained under heterotrophic and mixotrophic conditions (Chen et al., 2011; Cheng et al., 2009; Li et al., 2007; Liang et al., 2009; Xiong et al., 2008). The nitrogen source and concentration in the medium used are also known two of the most crucial factors affecting the lipid content of microalgae (Hsieh and Wu, 2009; Yeh and Chang, 2011). Therefore, it is of importance to utilize appropriate cultivation conditions and medium composition (especially nitrogen source and concentration) to achieve the best lipid production performance of microalgae species.

In this study, an indigenous microalgal isolate (identified as *Chlorella vulgaris* ESP-31) was examined with regard to its effectiveness for biomass and lipid production under different medium and cultivation conditions. To identify the lipid production performance of *C. vulgaris* ESP-31, the lipid content and lipid productivity were monitored with respect to time when the

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microalga was grown under different medium and cultivation conditions. The fatty acid profile of the microalgal lipid was also determined to evaluate the feasibility of using the produced microalgal lipid for biodiesel synthesis. This study aimed to identify the best conditions for the cell growth and lipid accumulation of *C. vulgaris* ESP-31, and to evaluate the potential of using the microalgal biomass thus produced as biodiesel feedstock.

2. Methods

2.1. Microalga strain and culture medium

C. vulgaris ESP-31 was isolated from a shrimp-culturing pond located in South Taiwan. The freshwater sample collected from Southern Taiwan was inoculated into BG11 medium and grown at 25 °C in an artificial light cabinet under illumination with fluorescent lamps (60 μmol/m^2 s, light/dark cycle: 16/8 h). The culture was then spread on BG11 medium agar plates and the appeared colonies were subcultured into the same medium until the pure strain was obtained (Ho et al., 2010).

The identity of the microalgal isolate was determined through plastid 23S rRNA gene sequence analysis. The genomic DNA of the microalga was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA). The plastid 23S rRNA gene was amplified by polymerase chain reaction (PCR) using two universal algal primers reported previously (Ho et al., 2010). The sequence analysis was conducted using a DNA sequencer (ABI Prism 310; Applied Biosystems) and the DNA sequences were assembled using the Fragment Assembly System program from the Wisconsin package version 9.1. The sequences of the microalgal strains were compared against plastid 23S rRNA gene sequences available from the GenBank databases. Multiple sequence alignment including microalgal strains and their closest relatives was performed using BioEdit software and MEGA4 (Ho et al., 2010).

The medium used for the preculture of *C. vulgaris* ESP-31 was Basal medium (Shi et al., 1997) that consisted of (g/l): KNO_3, 1.25; KH_2PO_4, 1.25; $MgSO_4{\cdot}7H_2O$, 1; $CaCl_2$, 0.0835; H_3BO_3, 0.1142; $FeSO_4{\cdot}7H_2O$, 0.0498; $ZnSO_4{\cdot}7H_2O$, 0.0882; $MnCl_2{\cdot}4H_2O$, 0.0144; MoO_3, 0.0071; $CuSO_4{\cdot}5H_2O$, 0.0157; $Co(NO_3)_2{\cdot}6H_2O$, 0.0049; EDTA·2Na, 0.5. The microalga was regularly grown at 25 °C for 4–5 days with a continuous supply of 2% CO_2 at an aeration rate of 0.2 vvm. The microalga culture was illuminated all day with a light intensity of ca. 60 μmol/m^2 s.

2.2. Operation of photobioreactor and culture conditions

The photobioreactor (PBR) used to cultivate *C. vulgaris* ESP-31 was a 1-liter glass vessel (15.5 cm in length and 9.5 cm in diameter) equipped with an external light source (14 W fluorescent light (TL5)) mounted on both sides set at 20-cm from the PBR (Yeh et al., 2010). The light intensity on the PBR was adjusted to ca. 60 μmol/m^2 s. Seed culture of *C. vulgaris* ESP-31 was inoculated into the reactor with an inoculum size of 20 mg/l. The reactor was operated at 25 °C, pH 6.2, 150 rpm agitation with CO_2 aeration (2%, 0.2 vvm). During microalgal growth, the liquid sample was collected from the sealed glass vessel with respect to time to determine microalgal biomass concentration, pH, residual nitrogen concentration and lipid content of the microalgal biomass.

The microalga was grown in the PBR on three different media (namely, Basal medium, Modified Bristol's medium CZ-M1 (Ip and Chen, 2005) and MBL medium). The compositions of these media are given in Table 1. Although the nitrogen source of the three media was the same (i.e., nitrate), the nitrate concentration in Basal, Modified Bristol's and MBL medium was 0.766, 0.547 and 0.062 g/l, respectively. The nitrate concentrations of the former two are about 10 times higher than that of the latter, thus the MBL medium is considered a nitrogen-poor one, while the other two are considered nitrogen rich. As for the trace metal ions, the three media contain similar trace elements (i.e., Fe, Zn, Mn, Mo, Cu, Co). However, since the concentration of trace metal ions in Basal medium is 10–6000 times higher than those of the other two, Basal medium is considered nutrient rich, while the other two are considered nutrient poor.

Table 1
Compositions of Basal medium, Modified Bristol's medium CZ-M1 and MBL medium.

Compositions	Basal medium (g/l)	Modified Bristol's medium (CZ-M1) (g/l)	MBL medium (g/l)
Medium type	Nitrogen rich	Nitrogen rich	Nitrogen poor
	Nutrient rich	Nutrient poor	Nutrient poor
NaCl		0.025	
$CaCl_2{\cdot}2H_2O$	0.1106	0.025	0.0368
$NaNO_3$		0.75	0.085
KNO_3	1.25		
$MgSO_4{\cdot}7H_2O$	1	0.075	0.037
$NaHCO_3$			0.0126
K_2HPO_4		0.075	0.0087
KH_2PO_4	1.25	0.175	
$Na_2O_3Si{\cdot}9H_2O$			0.0284
$FeSO_4{\cdot}7H_2O$	0.0498		
$FeCl_3{\cdot}6H_2O$		0.005	0.00315
EDTA·2Na	0.5		0.00436
H_3BO_3	0.1142	0.000061	0.001
$MnCl_2{\cdot}4H_2O$	0.0144		0.00018
$MnSO_4{\cdot}7H_2O$		0.000169	
$ZnSO_4{\cdot}7H_2O$	0.0882	0.000287	0.000022
$Na_2MoO_4{\cdot}2H_2O$	0.0119		0.000006
$(NH_4)_6Mo_7O_{24}{\cdot}7H_2O$		0.00000124	
$CuSO_4{\cdot}5H_2O$	0.0157	0.0000025	0.00001
$Co(NO_3)_2{\cdot}6H_2O$	0.0049		
$CoCl_2{\cdot}6H_2O$			0.00001

At the same time, different carbon sources were also used for the growth of *C. vulgaris* ESP-31. Inorganic carbons ($NaHCO_3$ and CO_2) were used as the carbon source in photoautotrophic cultivation, while organic carbon (glucose) was used in heterotrophic and photoheterotrophic cultivation. Mixotrophic cultivation, which means the microalgae could undergo photosynthesis and simultaneously use both organic and inorganic carbon as carbon sources, was also investigated in this study. The effects of these cultivation conditions on microalga growth and lipid production were investigated.

2.3. Determination of microalgal biomass concentration

The biomass concentration of the culture in the photobioreactor was monitored regularly by optical density measurement at a wavelength of 688 nm (i.e., OD_{688}) using a spectrophotometer (model U-2001, Hitachi, Tokyo, Japan) after appropriate dilution with deionized water. The OD_{688} values were converted to dry cell weight (DCW) concentration via appropriate calibration, as indicated below.

Under phototrophic and heterotrophic conditions:

$$\text{Biomass concentration (g dry cell/l)} = 0.1999 \times OD_{688} \quad (R^2 = 0.9073)$$

Under photoheterotrophic and mixotrophic conditions:

$$\text{Biomass concentration (g dry cell/l)} = 0.2917 \times OD_{688} \quad (R^2 = 0.9352)$$

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