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Strategies for enhancing lipid production from indigenous microalgae isolates

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ABSTRACT

Microalgae are widely regarded as the most promising source of green and sustainable fuel for the future. Compared to biofuels from terrestrial crops, the benefits of microalgal biofuels include high lipid content, rapid growth rate, high productivity per unit land area, and the ability to utilize wastewater for growth. In this study, five indigenous microalgae from Taiwan were selected to determine their potential for lipid production, namely *Chlamydomonas* sp. Tai-01, Tai-03 and Pin-01, as well as *Scenedesmus* sp. ESP-05 and ESP-07. Tai-03 proved to be the best strain, achieving an oil content and oil productivity of 28.6% and 124.1 mg/L/d, respectively. This was attained by inoculating 0.12 g/L Tai-03 into BG-11 medium with 25% initial nitrate concentration and LED light intensity of 200 μmol/m^2/s. The fatty acid methyl esters (FAMEs) obtained from the Tai-03 strain consisted mainly of palmitic acid, oleic acid and linoleic acid, making this microalga a suitable feedstock for biodiesel synthesis.

1. Introduction

Fossil fuels have provided us with a very useful energy source, and they are one of the pillars supporting our modern society and industries. However, we are depleting fossil fuels at an alarming rate, at the same time causing undesirable environmental pollution and global warming [1]. Therefore, many government and private bodies have been focusing on alternative energy sources that are renewable, sustainable and environmentally friendly. In this area, microalgae have presented itself as a promising green energy source. Compared to using first generation (oil palm, soybean, rapeseed, sugar cane) and second generation biofuels (lignocellulosic biomass, agricultural wastes) [2], generating biofuels from microalgae has several benefits: (1) high oil content (ranging from 20–70%), (2) higher yields per unit area of land, (3) utilize non-arable land and (4) can be cultured with wastewater and flue gas [3]. Microalgae also have the ability to produce a wide range of biofuels, including biodiesel [4], bioethanol [5], biohydrogen [6], syngas [7] and bioelectricity [8].

Biodiesel is a popular choice of microalgal biofuel, due to its potential utilization in existing diesel vehicles with little to no modifications, and compatibility with current fuel distribution infrastructure. In addition, biodiesel is also a cleaner fuel with relatively high energy density compared to diesel [9]. Biodiesel is acquired from the transesterification of extracted lipids from microalgal cell bodies. The catalyst required for transesterification process can be acids [10], alkalis [11] or enzymes [12]. It is common to find microalgae containing 20–50% lipids in their cell bodies [13]. Nitrogen starvation is the most dominant factor that resulted in lipid and carbohydrate accumulation in microalgae. The lack of nitrogen source in the medium induces stress on the growth of microalgae and in response, the microalgae accumulate lipids and carbohydrates [14]. The high oil content and biomass productivity of microalgae make them a potential producer of biodiesel. The

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Table 1
Comparison of oil productivity with recent research in lab-scale cultures.

Strain	Medium	Oil content (% dry wt)	Oil productivity (mg/L/d)	Cultivation time (d)	Reference
Ankistrodesmus falcatus (UTEX 242)	3 N BBM	12.0	55.0	14	[31]
Chlamydomonas sp. JSC4	HSM with 3.5% sea salt	33.1	169.1	7	[32]
Chlorella sorokiniana CY1	20% Deep sea water-amended medium	61.0	189.3	12	[15]
Chlorella vulgaris UTEX 395	3 N BBM	57.0	67.0	14	[31]
Dunaliella tertiolecta UTEX LB 999	Erdschreiber's medium	23.4	11.4	8	[33]
Nannochloropsis sp.	f/2 medium	35.0	63.0	14	[31]
Scenedesmus sp.	3 N BBM	43.0	106.0	14	[31]
Scenedesmus rubescens	Seawater	31.6	107.8	10	[34]
Thalassiosira sp.	Enriched seawater medium	–	248.5	6	[35]
Chlamydomonas sp. Tai-03	BG-11 medium	28.6	124.1	8	This study

performance of recent research on microalgal biodiesel production is listed in Table 1.

In this study, five different indigenous microalgae strains from Taiwan were characterized in terms of their lipid contents and productivities. The strain with the highest oil productivity was chosen for optimization strategies, which included altering the initial nitrate concentrations, LED light intensity and initial cell concentrations. The information obtained from this study would be useful for evaluating the potential of using the strain as feedstock for the production of biodiesel.

2. Materials and methods

2.1. Microalgae strain and medium composition

The five microalgae strains used in this study were isolated from Taiwan. The microalgae were identified as *Chlamydomonas* sp. Tai-01, Tai-03 and Pin-01, followed by *Scenedesmus* sp. ESP-05 and ESP-07. The identities of the above microalgal strains were determined via 18S rDNA sequence alignment. The 18S rDNA sequences of each strain contained 1050 base pairs and were amplified by polymerase chain reaction (PCR) using the primers NS1 (GTA GTC ATA TGC TTG TCT C) and NS4 (CTT CCG TCA ATT CCT TTA AG). The phylogenetic trees of the five strains were drawn using the genetics analytical software MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0) and illustrated in Fig. 1.

The three media used to culture the microalgae strains were BG-11 medium, Basal medium and Bold's Basal medium (BBM). The composition of BG-11 medium is (g/L): $NaNO_3$, 1.5; K_2HPO_4, 0.03; $MgSO_4.7H_2O$, 0.075; Citric acid anhydrous, 0.006; Na_2CO_3, 0.02, $CaCl_2{\cdot}2H_2O$, 0.036; Ammonium iron(III) citrate, 0.006; EDTA.2Na, 0.001; H_3BO_3, 0.00286; $MnCl_2{\cdot}4H_2O$, 0.00181; $ZnSO_4{\cdot}7H_2O$, 0.000222; $Na_2MoO_4{\cdot}2H_2O$, 0.00039; $CuSO_4{\cdot}5H_2O$, 0.000079; $Co(NO_3)_2.6H_2O$, 0.000049.

The composition of Basal medium is (g/L): KNO_3, 1.25; KH_2PO_4, 1.25; $MgSO_4{\cdot}7H_2O$, 1; $CaCl_2{\cdot}2H_2O$, 0.1106; $FeSO_4{\cdot}7H_2O$, 0.0498; EDTA.2Na, 0.5; H_3BO_3, 0.1142; $ZnSO_4{\cdot}7H_2O$, 0.0882; $MnCl_2{\cdot}4H_2O$, 0.0144; $Na_2MoO_4{\cdot}2H_2O$, 0.0119; $CuSO_4{\cdot}5H_2O$, 0.0157; $Co(NO_3)_2.6H_2O$, 0.0049.

The composition of BBM is (g/L): K_2HPO_4, 0.075; KH_2PO_4, 0.175; $NaNO_3$, 0.25; NaCl, 0.025; $MgSO_4{\cdot}7H_2O$, 0.075; $CaCl_2{\cdot}2H_2O$, 0.025; EDTA, 0.05; KOH, 0.031; $FeSO_4{\cdot}7H_2O$, 0.00498; H_2SO_4, 0.001; H_3BO_3, 0.01142; $ZnSO_4{\cdot}7H_2O$, 0.001412; $MnCl_2.4H_2O$, 0.000232; $CuSO_4{\cdot}5H_2O$, 0.000252; $Co(NO_3)_2{\cdot}6H_2O$, 0.00008; $Na_2MoO_4{\cdot}2H_2O$, 0.000192.

2.2. Determination of microalgae cell concentration

The cell concentration of the culture in the photobioreactor was determined 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 proper dilution with deionized water. The dry cell weight of the microalgae biomass was obtained by filtering 10 ml aliquots of culture through a cellulose acetate membrane filter (0.45 μm pore size, 47 mm in diameter). Each loaded filter was dried at 105 °C until the weight was invariant. The dry weight of the blank filter was subtracted from that of the loaded filter to obtain the microalgae dry cell weight (DCW). The OD_{688} values were converted to biomass concentration via calibration between OD_{688} and dry cell weight.

2.3. Determination of oil/lipid content

After appropriate cell growth, the microalgae cells were harvested from the culture broth by centrifugation (9000 rpm for 10 min). The cells were washed twice with deionized water, lyophilized, and weighed. The lipid composition was determined as fatty acid methyl esters (FAMEs) through the direct transesterification method described by Lepage and Roy [15]. The sample was analyzed by gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). Samples were injected into a 30 m long capillary column (Type no. 260M143P, Thermo Fisher Scientific, Waltham, MA, USA) with an internal diameter of 0.32 mm. Helium was used as the carrier gas, with a flow rate of 1.3 ml/min. The temperatures of the injector and detector were set at 250 and 280 °C, respectively. The oven temperature was initially set at 110 °C, increased from 150 to 180 °C at a rate of 10 °C/min, 180–220 °C at a rate of 1.5 °C/min, 220–260 °C at a rate of 30 °C/min and held at 260 °C for 5 min.

2.4. Operation of photobioreactor

The indoor photobioreactor (PBR) was a 1 L round glass vessel which was continuously illuminated with external LED light sources (DanceLight, Jan Cheng Lighting Co. Ltd., Taiwan) mounted on two opposite sides of the PBR. The microalgae were grown at room temperature of 24–26 °C and an agitation speed of 300 rpm. The light intensity on the vessel wall of the PBR was adjusted to ca. 200 $\mu mol/m^2/s$ using a LI-250 light meter with a LI-190SA pyranometer sensor (LI-COR, Inc., Lincoln, Nebraska, USA). The microalgae were pre-cultured and inoculated into the PBR with an inoculum size of 0.06 g/L. Air was filtered (0.22 μm) and mixed with CO_2 to give a CO_2 concentration of 2.0%. The culture broth was aerated continuously at a rate of 200 mL min^{-1} (0.2 vvm, volume gas per volume media per min). LED was chosen as the external light source because LED consumes less electricity and has uniform light intensity across the length of the light tube compared to fluorescent lamps [16]. CO_2 gas was chosen to imitate the utilization of flue gases from industries for mass culture of microalgae. All cultures were done in batch condition and all experiments were done in duplicates.

During cultivation, the initial pH for BG-11 medium was 7.0, Basal medium was 6.3, and BBM was 6.40. The pH was