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Characterization of photosynthetic carbon dioxide fixation ability of indigenous *Scenedesmus obliquus* isolates

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ABSTRACT

Photosynthetic mitigation of CO_2 with microalgae is promising from many aspects, thereby becoming a popular research topic. In this study, seven out of twenty-two indigenous *Scenedesmus obliquus* isolates obtained from southern Taiwan were selected for detailed study on their CO_2 fixation ability. Among them, 2 strains, namely, *S. obliquus* CNW-N and AS-6-1, displayed high cell growth rate and CO_2 removal ability when they were grown on 20% CO_2. The 2 strains show a high specific growth rate of 1.019 and 1.065 d^{-1}, respectively, along with a high biomass concentration (2.63 and 1.90 g L^{-1}, respectively). The biomass productivity of *S. obliquus* CNW-N and AS-6-1 was 201.4 and 150.7 mg L^{-1} d^{-1}, respectively and the CO_2 consumption rate could reach 390.2 and 290.2 mg L^{-1} d^{-1}, respectively, which are higher than that reported by most relevant studies.

1. Introduction

Since industrialization, carbon dioxide emission has kept increasing dramatically and is predicted to reach over 26 billion tons by the year of 2100 [1]. To minimize greenhouse gas emission at an acceptable, safe and reliable level, the Kyoto Protocol was ratified by over 170 countries in 1997, aiming to reduce greenhouse gases emission and prohibit the fast-growing global warming [2]. Taiwan Environmental Protection Administration (TEPA) reported that the CO_2 emission of Taiwan ranked 21st in the world in 2008 with a total CO_2 emission of over 260 million tons. Moreover, United Nations is going to impose carbon credit since 2010 and it is estimated that the carbon prices would reach US $270/ton [3]. For this reason, identifying an essential long-term strategy to mitigate carbon dioxide emissions is of great urgency.

Recently, many research and development efforts are implemented to reduce CO_2 emissions. Various methods have been applied to reduce CO_2 emissions; for instance, washing with alkaline solutions [4], multiwalled carbon nanotubes [5], amine coating activated carbon [6], etc. It is a nature process that CO_2 is removed through photosynthesis of all terrestrial plants and tremendous photosynthetic microorganisms on earth. However, terrestrial plants are expected to contribute only 3–6% of reduction in CO_2 emission [7]. In contrast, photosynthetic CO_2 fixation by microalgae and cyanobacteria are thought to be a feasible technology with environmentally friendly and energy-saving nature. Microalgae and cyanobacteria can grow much faster than terrestrial plants and the CO_2-fixation efficiency of algae is about 10–50 times faster than terrestrial plants [8]. Moreover, engineering approaches can be applied to further enhance the efficiency and commercial feasibility of CO_2 fixation by microalgae and cyanobacteria. In addition, the biomass of microalgae and cyanobacteria produced through the process of CO_2 fixation could be converted into a variety of biofuels or nutritious foods, etc., representing additional benefits from the microalgal CO_2 reduction process [9]. A variety of microalgae species have been applied in CO_2 reduction, including *Botryococcus braunii* [10], *Chlorella vulgaris* [11], *Scenedesmus* sp. [9], *Chlamydomonas reinhardtii* [12] and *Spirulina* sp. [9]. However, in most of those studies, CO_2 fixation ability was not characterized quantitatively, which is essential for engineering design of practical CO_2 biofixation processes.

Flue gases from power plants and industrial factory are always the first important donor of carbon dioxide emissions [3] and typically contain 15–20% carbon dioxide estimated by the IPCC criteria

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Table 1
The microalgae isolates collected from different locations. Related microalgae in GenBank are noted with 23S rDNA sequence similarity.

Strain	Location	Cell morphology	Closest relatives in GenBank	% Similarity
CNW-1	Niaosung Wetland	Oval	*Scenedesmus obliquus*	98
AS-6-1	NCKU	Oval	*Scenedesmus obliquus*	96
CNW-N	Niaosung Wetland	Fusiform	*Scenedesmus obliquus*	97
ESP-7	Pingtung shrimp pool	Fusiform	*Scenedesmus obliquus*	96
ESP-5	Pingtung shrimp pool	Fusiform	*Scenedesmus obliquus*	96
AS-F-7-1	NCKU	Fusiform	*Scenedesmus obliquus*	98
FSP-3	Pingtung shrimp pool	Oval	*Scenedesmus obliquus*	95

[13]. Therefore, in this study, microalgae species possessing high potential for application in biofixation of CO_2 were isolated. The CO_2 biofixation ability of the isolated microalgae strains were characterized under a carbon dioxide concentration of 20% in terms of the CO_2-fixation efficiency and microalgae biomass production performance. The aim of this work was to identify effective microalgae strains for future uses in developing practical microalgae-based CO_2 reduction process.

2. Materials and methods

2.1. Isolation and identification of microalgae

Microalgae strains were isolated from freshwater area located in southern Taiwan. For microalgae isolation, the freshwater sample was inoculated into BG11 medium [14] and grown at 25 °C in an artificial light cabinet under illumination with fluorescent lamps at 60 μmol m^{-2} s^{-1} on a light/dark cycle of 16 h/8 h. The culture was spread onto BG11 agar plates and colonies were subcultured into the same media until a culture dominated by the pure strain was obtained.

The identity of the microalgal isolates was determined through plastid 23S rRNA gene analysis. The DNA of microalgae 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 [15]. The sequences were acquired using a DNA sequencer (ABI Prism 310; Applied Biosystems) and the DNA sequences were then assembled using the Fragment Assembly System program from the Wisconsin package version 9.1 [16]. 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 [17] and MEGA4 [18]. Phylogenetic trees were inferred by using the maximum-parsimony [19] and neighbour-joining [20] algorithms. An evolutionary distance matrix was generated for the neighbour-joining algorithm by using the distance model [21]. Bootstrap analysis for the neighbour-joining tree was performed on the basis of 1000 resamplings.

2.2. Microalgae culture and medium composition

The microalgal species used in this work were obtained from freshwater located in southern Taiwan. The microalgae were identified as *Scenedesmus obliquus* by its morphology as well as by 23S rDNA sequence matching (details described in Table 1). A modified version of Detmer's Medium (DM) was used to grow the pure culture of *S. obliquus*. The medium consisted of (g L^{-1}): $Ca(NO_3)_2{\cdot}4H_2O$, 1.00; KH_2PO_4, 0.26; $MgSO_4{\cdot}7H_2O$, 0.55; KCl, 0.25; $FeSO_4{\cdot}7H_2O$, 0.02; EDTA·2Na, 0.2; H_3BO_3, 0.0029; $ZnCl_2$, 0.00011; $MnCl_2{\cdot}4H_2O$, 0.00181; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.000018; $CuSO_4{\cdot}5H_2O$, 0.00008. The *S. obliquus* strains were grown at 28 °C for 12 days (or 288 h) under a light intensity of approximately 60 μmol m^{-2} s^{-1} (illuminated by TL5).

2.3. Operation of photobioreactor

The photobioreactor (PBR) was a 1-L glass-made vessel illuminated with an external light source (14 W TL5 tungsten filament lamps; Philips Co., Taipei, Taiwan) mounted on both sides. The light intensity on the vessel wall of PBR was adjusted to ca. 60 μmol m^{-2} s^{-1}. The seven *S. obliquus* strains were pre-cultured and inoculated into the photobioreactor with an inoculum size of 15–18 mg L^{-1}. The PBR was operated at 28 °C, pH 6.2, and an agitation rate of 300 rpm. Serving as the sole carbon source, carbon dioxide (20%) was fed into the culture continuously for all strains during cultivation for 12 days. The liquid sample was collected from the sealed glass vessel with respect to time to determine microalgae cell concentration, pH and residual nitrate concentration.

2.4. Determination of microalgal cell concentration

The cell concentration of culture in the photobioreactor was determined regularly by measuring optical density at wavelength 685 nm (denotes as OD_{685}) using UV/VIS spectrophotometer (model U-2001, Hitachi, Tokyo, Japan) after proper dilution with deionized water to give an absorbance range of 0.05–0.9. The dry cell weight (DCW) of microalgae biomass was obtained by filtering 50 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 blank filter was subtracted from that of the loaded filter to obtain the microalgae dry cell weight. The OD_{685} values were converted to biomass concentration via proper calibration between OD_{685} and dry cell weight and the conversion factor was determined (i.e., 1.0 OD_{685} approximately equals to 0.22–0.68 mg DCW L^{-1} depending on different strains).

2.5. Measurement of light intensity

The light intensity on the reactor wall was measured by a LI-250 Light Meter with a LI-190 quantum sensor (LI-COR, Inc., Lincoln, NE, USA). This light meter gives a unit of μmol m^{-2} s^{-1} for the measured light intensity.

2.6. Measurement of residual nitrate content

Nitrate concentration was determined according to the modified method reported by Collos et al. [22]. A liquid sample collected from photobioreactor was filtered by 0.22 μm pore size filter and then diluted 20 fold with DI water for each sample. The sample was collected and residual nitrate content was determined according to optical density at wavelength of 220 nm (i.e., OD_{220}) using UV/VIS spectrophotometer (model U-2001, Hitachi, Tokyo, Japan).

2.7. Determination of growth kinetic parameters

Time-course profile of the biomass concentration (X; g L^{-1}) was used to calculate the maximum specific growth rate (μ_{max}, d^{-1}). The maximum biomass concentration achieved in the PBR was