Applied Energy 96 (2012) 371–377

Contents lists available at SciVerse ScienceDirect

Applied Energy

journal homepage: www.elsevier.com/locate/apenergy

Characterization of microalga *Nannochloropsis* sp. mutants for improved production of biofuels

Kandiah Anandarajah [a], Guruvaiah Mahendraperumal [a,b,*], Milton Sommerfeld [a], Qiang Hu [a]

[a] Laboratory for Algae Research and Biotechnology (LARB), Arizona State University Polytechnic Campus, 7001 E. Williams Field Road, Mesa, AZ 85212, USA
[b] Department of Biology, Brooklyn College of CUNY, 2900 Bedford Ave, Brooklyn, NY 11210, USA

ARTICLE INFO

Article history:
Received 18 November 2011
Received in revised form 17 February 2012
Accepted 21 February 2012
Available online 26 April 2012

Keywords:
Microalgae
Total lipid
Fatty acids
Mutant
Biofuel
Ethylmethanesulfonate

ABSTRACT

To select microalgae with a high biomass, chlorophyll *a*, lipid, and fatty acid content of two mutants (LARB-202-2 and LARB-202-3) and their parent wild type of the unicellular green alga *Nannochloropsis* sp were cultured with air containing 1% CO_2 for a week in 1 L bubbled tubes with continuous illumination at 140 or 300 μmol photons m^{-2} s^{-1}. Overall biomass productivity of all three strains were higher under high light (HL, 300 μmol photons m^{-2} s^{-1}) with LARB-202-3 achieving the highest volumetric productivity (0.9 g L^{-1} d^{-1}) and parent wild type the lowest (0.72 g L^{-1} d^{-1}) when cultured for 6 days with medium nitrogen level. Biomass productivity of all three strains were substantially low in response to low light (LL, 140 μmol photons m^{-2} s^{-1}) growth conditions. However, LARB-202-3 showed the highest biomass productivity (0.74 g L^{-1} d^{-1}) under LL conditions too. LARB-202-3 possessed high photosynthetic productivity as measured by chlorophyll *a* (Chl *a*) content under HL conditions throughout the growth period. The content of Chl *a* declined gradually in all three tested strains over time. Volumetric productivity of biomass was closely associated with the cellular content of Chl *a*. Total lipid productivity of LARB-202-2 and LARB-202-3 grown in low nitrogen media for 12 days were 273 and 297 mg L^{-1} d^{-1}, respectively, while that of wild type parent was 244 mg L^{-1} d^{-1}. Major medium chain fatty acids (e.g. C14:0 and C16:0) make up nearly 63% of total fatty acids in all three strains. No significant variation in major fatty acid composition was found among the tested mutants and the wild type parent. The mutants are discussed in terms of their comparative advantage over their wild type parent with respect to their potential utilization by the algal biotechnology industry for the production of biofuels.

Published by Elsevier Ltd.

1. Introduction

It is well recognized that renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Biodiesel derived from oil crops is a potential renewable, carbon neutral alternative to petroleum fuels. Unfortunately, biodiesel derived from oil crops, waste oil and fat cannot realistically satisfy even a small fraction of the existing demand for carbon neutral alternative to petroleum fuel [1]. Microalgae have been considered as an alternative source of renewable biodiesel and bio-jet fuel due to their ability to produce substantial amount of triacylglyceride (TAG), rapid growth potential with shorter generation time, and tolerance to environmental conditions [2,1,3].

Production of algal oil requires an ability to inexpensively produce large quantities of oil-rich microalgal biomass. Cost of producing microalgal biodiesel can be reduced substantially by using modern production strategy, and improving capabilities of microalgae through genetic engineering. Genetic and metabolic engineering are likely to have the greatest impact on improving the economics of production of microalgal diesel [4,5]. At present, induced mutagenesis presents a substantial advantage compared to genetic engineering [6]. This is not reported for most microalgae species [7]. However, following exposure to mutagens, mutations in a particular gene might occur, at most, with an incidence of 10^{-3}. Thus, mutant isolation requires the use of selection and/or screening techniques that can reveal or identify the rare colonies arising from mutant cells [8].

Currently only a few microalgae are being considered or are already being exploited for the production of biofuel. *Nannochloropsis* sp is one of them. *Nannochloropsis* is a marine unicellular alga belonging to the Eustimatophyceae and are attractive for commercial exploitation. They grow fast, typically doubling their biomass once a day and also possess high oil productivities that are desired for producing biodiesel. Mutation studies on *Nannochloropsis oculata* have led to the improvement xanthophylls [9] Eicosapentaenoic acid (EPA) and Docosahexaenoic (DHA) acids [10]. However, no mutants of the genus *Nannochloropsis* have been identified and employed to date with improved characteristics to

* Corresponding author. at: Department of Biology, Brooklyn College of CUNY, 2900 Bedford Ave, Brooklyn, NY 11210, USA. Tel.: +1 347 784 5619.
E-mail address: mahendraperumal@gmail.com (G. Mahendraperumal).

0306-2619/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.apenergy.2012.02.057

enhance biodiesel production. Biomass growth and biochemical analysis on lipid content suggest that enhance biomass growth rate, increased oil content in biomass, and increased photosynthetic efficiency to support biomass growth are essential for successful introduction and commercialization of biofuel with the use of microalgae.

The lipids from microalgae could be used in different processes for energy exploitation; the best possible use is certainly its transformation to a biofuel, especially biodiesel [1]. Detailed lipid analyses of relatively few species of green algae have been carried out, mainly of those that have been widely used as model systems for biochemical studies. For example, most of these present fatty acid compositions are similar to that of higher plants, characterized by predominance of C16 (Palmitic acid methyl ester) and C18 (Stearic acid methyl ester) fatty acids [11]. Lipid content and fatty acid compositions of any algae species are primary determinants for producing biofuel and also other pharmaceutical or any other useful industrial products.

Microalgae undergo significant physiological and chemical changes in response to variations in light, temperature, and nutrient availability [12,13]. Microalgae grown at various irradiance levels display a significant change in their gross chemical composition, pigment content and photosynthetic activity [14,15]. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll *a* and this is the molecule which makes photosynthesis possible. The different light sources and intensities were studied in potential of microalgae oil from *Dunaliella* sp. as a feedstock for biodiesel production [17]. The growth of *Nannochloropsis* sp. was enhanced by aeration with 15% CO_2. Lipid production of *Nannochloropsis* sp. was enhanced by the two-phase cultivation [18].

When microalgal strains were tested for their lipid production potential by evaluating biomass productivity and lipid content in laboratory cultures, the biomass productivity ranged from 0.04 to 0.37 g L^{-1} d^{-1} and lipid productivity ranged from 17 to 61 mg L^{-1} d^{-1} [14]. The total content of lipids in microalgae may vary from about 1% to 85% of the dry weight [2] with values higher than 40% being typically achieved under favorable nutrient limitation. Factors such as irradiance, temperature and, most markedly, nutrient availability have been shown to affect both lipid composition and lipid content in many algae [19,3]. In this work, we report isolation and characterization of two mutants that were produced after mutating the existing wild type *Nannochloropsis* sp. culture after testing a fairly large number of the mutants. The selected strains were tested on a large volume of culture along with the parent wild type.

This work is a first attempt to increase biomass production and lipid content with the use of chemically induced mutants produced from wild type *Nannochloropsis* sp. In this study we report the characterization of mutants selected from chemical mutagenesis of wild type *Nannochloropsis* sp.

2. Materials and methods

2.1. Preparation of culture media for mutation experiment

The parent culture of wild type *Nannochloropsis* sp was selected from LARB (Laboratory for Algae Research and Biotechnology, Arizona State University, USA) collection based on increased biomass production. Cells were cultured aseptically in sea water base (35 g L^{-1} sea salt mix manufactured by Oceanic) on modified Guillard f2 medium [20] enriched with $NaNO_3$ (8.82 mM), NaH_2PO4 (0.22 mM), four times the amount of trace metal mix, and vitamin mix reported in Guillard f/2 medium and maintained at 22 ± 1 °C under 24 h cool white fluorescent lights at 50–60 μmol photons m^{-2} s^{-1} irradiance. The culture was grown until early log-phase (3×10^7 cells mL^{-1}) and was mutagenized at this stage.

2.2. EMS treatment and survival count

The mutation program used ethyl methane sulfonate (EMS) solution as mutagenic agent. The first step of the mutation program was determination of appropriate concentration of EMS and duration of mutagenic treatment of the parent culture, so as to guarantee a successful strain improvement methodology; for that purpose, survival rate under EMS treatment was obtained.

Preliminary analysis of several survival data with EMS concentration ranging from 25 to 100 μL mL^{-1} (approximately 230–920 mmol) showed that EMS treatment with concentration 50 μL mL^{-1} could produce wide range (0.18–87%) of survival rate depending on the duration of EMS treatment. The effects of EMS treatment duration on cell survival and conversion rate to colonies were studied after EMS treatment with 50 μL mL^{-1} concentrations. To mutate algal cells and also to observe effects of EMS, aseptically grown algal cells were transferred to test tubes containing potassium buffer, pH of 7 at the end of the exponential growth stage (approximately 3.0×10^7 cells mL^{-1}). The cells were treated with EMS concentration 50 μL mL^{-1} in a screw-cap glass tube. After incubating the treated cells with different time intervals (20–180 min) at 30 °C, the mutagenic reaction was stopped at each time point by adding 7% solution (w/v) of sodium thiosulphate. The individually treated cell cultures were thoroughly washed twice with distilled water and incubated in the dark over night prior to plating. Suitable dilutions were made with f/2 medium. Aliquots of the mutagenized cultures were spreads on to enriched f/2 media solidified with 1% agar (Bacto™ agar) in petri dishes. Conversion of treated cells into colonies was monitored. Colonies were counted after 6 weeks from the time of plating. Fast growing colonies were individually inoculated into the enriched f/2 liquid medium in test tubes and grown until early log-phase. Thus, fast growing mutant strains were cultured to grow for two generations following treatment with the mutant before screening. The cultures were then transferred to 250 mL (column inner diameter 29 mm, height 60 cm) tubular columns bubbled with 1% CO_2 contained in air and illuminated continuously at light intensity of 80 μmol photons m^{-2} s^{-1} on the surface of the outer tube with cool-light fluorescent bulbs and cultured in enriched f/2 media until it reached mid-log phase for further screening.

2.3. Screening of mutant strains

Two mutant strains have been successfully isolated from screening. The two mutant strains (LARB-202-2, LARB-202-3) and their parent wild type *Nannochloropsis* sp were selected and tested for their growth and lipid production. Each strain culture with initial density of 0.5 g was grown in enriched f/2 medium in 1.0 L cylindrical glass columns (column inner diameter 50 mm, height 60 cm). The culture was bubbled with 1% CO_2 and air and illuminated continuously at light intensity of 80 μmol photons m^{-2} s^{-1} on the surface of the outer tube with cool-light fluorescent bulbs and grown until it reaches mid-log phase. Cultures at mid-log phase were used as inoculumn and transferred to the 1.0 L glass columns for indoor experimental studies.

2.4. Culture conditions

Inoculums density was adjusted to have 1.0 g L^{-1} cell density. To examine the physiological consequences of changes in the lipid production and fatty acid compositions, we compared the rates of growth of LARB-202-2 and LARB-202-3 mutants and their parent

Download English Version:

https://daneshyari.com/en/article/243283

Download Persian Version:

https://daneshyari.com/article/243283

Daneshyari.com