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Enhancement of lipid production in *Scenedesmus* sp. by UV mutagenesis and hydrogen peroxide treatment

Ramachandran Sivaramakrishnan, Aran Incharoensakdi *

Laboratory of Cyanobacterial Biotechnology, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

HIGHLIGHTS

- UV mutagenesis of *Scenedesmus* sp. can increase both biomass and lipid content.
- Oxidative stress by H_2O_2 in the UV mutant improves the total lipid production.
- FTIR results confirms the changes of lipid and protein content in H_2O_2 treated mutant.
- UV mutagenesis and H_2O_2 treatment do not affect the quality of methyl ester.

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ABSTRACT

The high potential UV mutagenized *Scenedesmus* sp. was obtained in which the cells had a higher biomass and lipid content than the wild type with an increase from 1.9 to 2.4 g/L and from 40 to 55% of dry cell weight respectively after 12 days. Oxidative stress imposed by H_2O_2 treatment decreased the biomass of both the wild type and the mutant. The H_2O_2 treated mutant when grown in BG11 medium showed an increase in biomass which was in contrast to a decreased biomass observed in the H_2O_2 treated wild type. A 3-fold increase in lipid yield of 1.63 g/L was obtained in the oxidative stress-induced mutant compared to the wild type. Overall results indicate that prior treatment of UV-mutagenized *Scenedesmus* with oxidative stress can increase the total lipid production which, due to its derived methyl ester having acceptable biodiesel properties, can be potentially utilized for biodiesel production.

1. Introduction

The rise in carbon emission and the depletion of fossil fuels have triggered the concern to search for renewable biofuels. In recent years, particularly biodiesel has received much attention. To produce biodiesel, microalgae have been considered one of the best sources for biodiesel production because of their good characteristics as feedstock (Hu et al., 2008). The cultivation of microalgae requires few arable lands and produces up to 10–20 times production per hectare (Thompson et al., 1990). Several microalgae produce high quality and quantity of lipids which is very suitable for biodiesel production. Thus, high lipid content and rapid cell growth is critical for the scale up process (Bougaran et al., 2012). Screening of high lipid content microalgae with high biomass is the priority and foundation for successful commercialization of biodiesel from microalgae (Zhang et al., 2010). To increase the productivity of lipid in the cells, strain improvement by mutagenesis is an effective method. Generally, mutagenesis in microalgae is performed by a physical method (X-rays, Gamma and UV rays) (Zayadan et al., 2014) and by a chemical method (ethyl methane sulfonate (EMS), nitrosomethyl guanidine) (Bird and Neuffer, 2015). Chemical mutagenesis approaches have the safety problem to the operator and the environment, whereas UV mutagenesis is rapid, effective and safe to the environment (Fang et al., 2013). To increase the microalgae lipid in a large-scale cultivation, UV radiation is the promising approach. Moreover, UV radiation has genetically and physiologically deleterious effects leading to metabolic changes in microalgae (Guihéneuf et al., 2010). Compared to UV-A or B, UV-C has more energetic radiation and it is believed to have efficient effects on microalgae (Sharma et al., 2014). Hence, UV mutagenesis can achieve higher benefit and cause significant effects on the organism. UV-C radiation together with the nutrient stress stimulate the lipid production in *Tetraselmis* sp. (Sharma et al., 2014). Due to the increase of oil feedstock price, it is necessary to improve

* Corresponding author.
E-mail address: aran.i@chula.ac.th (A. Incharoensakdi).

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the strain with high lipid content. So far, the positive effect of UV mutagenesis on both biomass and lipid contents in microalgae have not been reported. Unlike nutrient stress, oxidative stress increases the lipid peroxidation in microalgae (Yilancioglu et al., 2014). Oxidative stress increases the reactive oxygen species (ROS) and thus enhances the lipid content. Oxidative stress by H_2O_2 was shown to increase the lipid content in the *Chlorella vulgaris* (Battah et al., 2014). However, to the best of our knowledge, no information is available concerning the improvement of both biomass and lipid content in various organisms including microalgae by UV mutagenesis and oxidative stress treatment.

To obtain microalgae with high lipid and biomass, the *Scenedesmus* sp. isolated from stone quarry pond water (India) was irradiated by UV. The obtained mutants were screened based on their biomass and lipid contents. The selected mutant was further subjected to oxidative stress by H_2O_2 treatment in which the treated mutant showed an increase of lipid yield.

2. Materials and methods

2.1. Organism and culture conditions

Scenedesmus sp. was isolated from stone quarry pond water of Vellore, India and its accession number was KR025877 (GenBank) (Sivaramakrishnan and Incharoensakdi, 2017a). The axenic strain was grown and maintained in BG11 medium at 27 ± 1 °C under continuous illumination (fluorescent white light) of 50 μmol photons/m^2/s with shaking at 100 rpm. Microscopic analysis was carried out daily to check the purity of the culture.

2.2. UV mutagenesis and screening

The 12 days grown *Scenedesmus* sp. culture (3 ml, OD_{730} = 1.0) was distributed in a 9-cm diameter petri-dish. Mutation was induced using a UV-C lamp with 253.7 nm (3.4 W m^{-2}. The distance between the petri-dish and the UV lamp was adjusted to 15 cm. The time of exposure was varied from 0 to 40 min. After irradiation 500 μl of the sample was transferred to sterilized centrifuge tubes and kept in the dark for 24 h to prevent photoreactivation. The samples of mutagenized cultures were spread on BG11 agar plates and incubated for 2–3 weeks. Mutant colonies were screened visually on the basis of their size. Thirty-four clearly visible colonies M1 to M34 were transferred to a liquid BG11 medium (Fig. S1). Simultaneously, these colonies were grown on BG11 petri-plates for several generations under the same growth conditions to ensure the cells survival. Furthermore, the best mutant was finally isolated based on its lipid content and biomass productivity. The cells were harvested by centrifugation at 2790g, 10 min, room temperature. The pellet was washed with fresh BG11 medium and inoculated into 100 ml fresh nutrient medium at an initial cell density of OD_{730} = 0.05 and grown on a rotary shaker at 160 rpm with continuous illumination (50 μmol photons/m^2/s) for 12 days and used for the analysis.

2.3. Hydrogen peroxide treatment

The 12 days grown cells were inoculated into BG11 medium supplemented with different hydrogen peroxide concentrations (0–5 mM) with the initial cell density of OD_{730} = 0.05. The H_2O_2 treated cells (12 days) were harvested by centrifugation and washed 3-times with distilled water. The treated cells were inoculated into fresh BG11 medium without H_2O_2 with the initial cell density of OD_{730} = 0.05, grown for 12 days and used for further analysis of dry cell weight (DCW), lipid and acetyl-CoA carboxylase (ACC). After centrifugation, the cell pellets were re-suspended with phosphate-buffer (pH 7.2) and sonicated at 100 W for 10 min. The supernatant after centrifugation was analysed for the acetyl-CoA carboxylase activity using ELISA kit (Life span biosciences).

2.4. Fatty acid and biodiesel properties analysis

The fatty acid compositions of isolated microalgae were analysed by gas chromatography as described (Sivaramakrishnan and Incharoensakdi, 2017b) in Section S.6. The biodiesel properties, such as saponification value (SV) and iodine value (IV) were determined according to Francisco et al. (2010). Cetane number (CN) and degree of unsaturation (DU) were estimated according to Ramos et al. (2009).

2.5. Statistical analysis

The results are presented as mean of three replicate values, with the error bars showing standard deviations (means ± SD, n = 3). Statistical significance ($p < 0.05$) was analysed by *t*-test comparisons using graph pad software.

3. Results and discussion

3.1. Survival rate of the mutagenic cells

As shown in Table S1, the microalgal cells were sensitive to UV irradiation. No cell survival was observed under long-time radiation (40 min). UV treatment affected the survival rate of *Scenedesmus* sp. cells. The increase of UV irrradiation time increased the cell death (fatality rate %). Increasing UV irradiation time from 5 to 40 min decreased the survival rate from 86.6 to 0%. The results indicated that the long-time UV irradiation was the most lethal. However, a few colonies appeared after 10 and 15 min UV irradiation. The survival ability of the microalgae was reduced after long-time exposure to UV irradiation. The severity of the DNA damage depends on the UV exposure time. The self-DNA repair mechanism was interrupted when kept in dark for 24 h. The UV mutation in *Chlorella* sp. resulted in high yield of biomass and lipid as well as the stability which is suitable for the biodiesel production (Liu et al., 2015). Zhang et al. (2016) studied the breeding of high biomass and lipid in *Desmodesmus* sp. (*Scenedesmus* sp.) using EMS mutagenesis. The mutants showed high biomass yield with high lipid production and relatively high stability rendering its suitability for further mutational studies. Therefore, suitable exposure limit is necessary for satisfactory mutagenesis and further selection was determined by screening process.

3.2. Screening of the mutant

Random mutagenesis is useful to achieve genetic and functional modifications of an organism. The screening of the mutant was done with respect to the lipid accumulation and DCW as shown in Fig. S1. From the thirty-four colonies, various positive mutants were obtained. Some UV mutagenized cells showed higher lipid content and some showed higher cell growth. However, the ultimate aim of this study is to find the mutant with high lipid content and biomass. Thus, among the thirty-four colonies (M1 to M34), the colony M22 obtained from the 10 min UV exposure plate showed the highest biomass and lipid contents. In the overall analysis of various mutants, many positive mutants were obtained from 10 min UV exposure plates. The longer than 10 min UV exposed plates produced negative colonies, i.e. low lipid content or low biomass content. The M16 strain showed highest biomass of 2.56 g/L DCW and its lipid content was 29% DCW, the highest value of 2.56 g/L DCW was possible with *Scenedesmus* sp. The DCW higher than 2.56 g/L was reported by Wu and Miao (2014)