Algal Research 26 (2017) 25–38

Contents lists available at ScienceDirect

Algal Research

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

Improved productivity and oxidative stress tolerance under nitrogen starvation is associated with the ablated Δ5 desaturation in the green microalga *Lobosphaera incisa*

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ARTICLE INFO

Keywords:
Arachidonic acid
LC-PUFA
Light stress
Triacylglycerol
Reactive oxygen species and nitrogen starvation

ABSTRACT

The green microalga *Lobosphaera incisa* deposits exceptional amounts of arachidonic acid (ARA, 20:4 *n*-6) in the storage lipid triacylglycerol (TAG) under nitrogen (N) starvation. The mutant P127, impaired in Δ5 desaturation, is devoid of ARA and produces dihomo γ-linolenic acid (DGLA, 20:3 *n*-6). Here, we performed a comparative assessment of the effects of N starvation in the mutant and wild type (WT) to elucidate the consequences of mutation on biomass and long-chain polyunsaturated fatty acid (LC-PUFA) productivity. The initial cell density and external light intensities were used as variables for altering incident light availability in the N-depleted cultures. The majority of examined parameters were impaired in the WT, in particular, upon increasing the magnitude of stress applied. Under high light, the highest biomass and LC-PUFA productivities were documented in the cultures of higher cell density in both strains, with maximal productivities attained by the mutant. We surmised that the high content of ARA in N-starved WT cells renders cellular lipids susceptible to reactive oxygen species (ROS), produced under such stress conditions and thus aggravates photosynthetic parameters and biomass production. This assumption was corroborated by the higher lipid peroxidation level during starvation and the lower glutathione content in the WT in the N-replete cells. The mutant also appeared to be more resistant to administration of oxidative stress-generators, methyl viologen and H_2O_2, than the WT. Furthermore, the expression of selected examined genes functioning in the redox status maintenance in the chloroplast was downregulated in the WT under N starvation. We conclude that the characteristically augmented oleic acid (18:1 *n*-9) accumulation in the TAG of P127, is associated with the decreased expression of LC-PUFA biosynthesis genes, leading in turn to alleviation of oxidative stress and improved DGLA productivity under N starvation. Hence, from the biotechnological stand-point, strategies avoiding oxidative damage are critically important for the WT *L. incisa* cultivation.

1. Introduction

The oleaginous microalga *Lobosphaera incisa* (Trebouxiophyceae, Chlorophyta), earlier known as *Parietochloris incisa* [1], was isolated from the slopes of a snow mountain in Japan. This habitat is characterized by a broad range of environmental fluctuations, especially in light and temperature. *Lobosphaera incisa* was found to accumulate high contents of the omega-6 long-chain polyunsaturated fatty acid (LC-PUFA), arachidonic acid (ARA; 20:4 *n*-6) [2,3] in its storage lipid triacylglycerol (TAG). A mutant strain P127 was isolated by chemical mutagenesis of the wild type (WT) [4]. Due to the nonsense mutation in the Δ5-desaturase gene, the mutant is unable to produce ARA, and therefore, its precursor dihomo-γ linolenic acid (DGLA; 20:3 *n*-6) is accumulated [4–6]. While TAG of the WT *L. incisa* is enriched in ARA, in particular under nitrogen (N) starvation, the alga can synthesize TAG enriched with C18 precursors, such as oleic acid (18:1 *n*-9) when the biosynthesis of ARA is inhibited [2]. It has been shown that the characteristics of a high amount of LC-PUFA in the storage lipid remunerates this alga by utilizing ARA to construct the membrane lipids when exposed to abrupt cold stress or during growth recovery from N starvation [2,7]. However, the impacts of LC-PUFA content in the cellular lipids of WT and P127 on survival and recovery from stresses are

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http://dx.doi.org/10.1016/j.algal.2017.06.026
Received 17 February 2017; Received in revised form 28 June 2017; Accepted 29 June 2017
Available online 07 July 2017

still not well explored.

The unusual ability of the *L. incisa* WT and the mutant P127 to produce high contents of ARA- and DGLA-rich TAG, respectively [2,8], makes these strains potent sources for production of two valuable omega-6 LC-PUFA by photo-biotechnology. Both these LC-PUFA are important for human health; being the essential *n*-6 LC-PUFA for brain and visual acuity development, ARA is commercially incorporated in infant formula [9,10]. The perspectives of mass production of DGLA is also gaining attention owing to its several health benefits such as anti-inflammatory, anti-thrombotic, anti-hypertensive, anti-allergic and anti-atherosclerotic effects [11–14].

Under N starvation, the fatty acid content in the WT can reach over 35% of dry weight (DW) whereas ARA constitutes over 50% of total fatty acids (TFA) with over 90% of cell ARA deposited in TAG [15]. Under similar conditions, the mutant can attain the fatty acid content of around 30–35% of DW with 30–35% DGLA of the TFA with the predominant fraction of the cell DGLA deposited in TAG [4,6,8]. P127 accumulates higher levels of monounsaturated fatty acid 18:1 *n*-9 in its TAG than that in the WT along with other featured differences in the cellular content and FA composition of different lipid classes [5]. The accumulation of ARA and DGLA in either strain under N starvation is affected by the availability and intensity of light, which are the factors of photosynthetic active radiation (PAR) and the culture density [16,17] or their combination [8].

Numerous studies have confirmed that alterations in irradiance and N availability promote remarkable changes in gross cellular chemical composition, such as protein and carbohydrate content, pigment and lipid content, FA composition, as well as photosynthetic activity of microalgae either alone or in combination with other stresses [17–26]. A general trend of lipid accumulation, particularly of TAG, in response to N deficiency has been observed in both categories of marine and freshwater algae [15,16,22,25,27,28]. However, the studies on physiological and molecular responses of algal cells rich in LC-PUFA-containing TAG upon alterations in environmental conditions are still limited e.g. [6,29].

High light stress alone can significantly affect the function of the photosynthetic apparatus by altering its electron transport system, redox status, antennae proteins and pigments content and composition and thereby affect the overall growth of algae. When reactive oxygen species (ROS) generation outpaces the scavenging rate, irreversible oxidative damage may be caused to macromolecules [30]. Such conditions can be generated under adverse environmental conditions such as nutrient and light stress that are common in microalgal cultures [25]. On the one hand, in oleaginous microalgae, induced fatty acid synthesis and accumulation of TAG under stressful conditions, deems to alleviate negative consequences of the over- reduced photosynthetic chain [17,31]. On the other hand, LC-PUFA producing microalgae such as *L. incisa* may have higher risk of membrane damage due to the higher susceptibility of lipid peroxidation of membrane and storage lipids.

In this report, we set on to comparatively evaluate the effects of cultivation conditions, in particular, the interactive effects of exposure to high light and N depletion in two *L. incisa* strains differing in their LC-PUFA composition. In our previous work [5], we reported the fatty acid composition of glycerolipids in both strains of *L. incisa* at the initial stages of N starvation and noted the difference between strains in the content of strain-specific LC-PUFA in all glycerolipid classes, including the major chloroplast lipid monogalactosyldiacylglycerol (MGDG). However, we could only speculate on the functional significance of ARA in the lipids of the WT strain and consequences of its substitution by DGLA in the mutant. In our current work, we focus on elucidation of the functional significance of ARA-deficiency in *L. incisa* mutant P127 under different stresses [32]. In the present work, we evaluated the effects of the decreased unsaturation level in the cellular lipids of the P127 compared to WT on biomass and LC-PUFA production and response to N starvation under different external light intensities, and under oxidative stress caused by administration of ROS-generators. Identifying the factors regulating LC-PUFA-rich lipid biosynthesis in *L. incisa* strains is of prime importance for optimizing culture conditions and maximizing lipid productivity. This research is of both basic and applied interest, since studies on the role of LC-PUFA in microalgae are very rare.

2. Materials and methods

2.1. Strains and culture conditions

The wild type (WT) *L. incisa* (SAG 2468) and its Δ5-desaturase mutant P127 were obtained from the Microalgal Biotechnology Laboratory, J. Blaustein Institutes for Desert Research. The strains were cultivated in the modified BG-11 (mBG-11) medium [33]. Experiments were conducted in 500 mL glass columns (3.8 cm internal diameter) placed in a temperature-regulated water bath at 25 °C and aerated by bubbling with a mixture of 2% CO_2 in air. Continuous illumination was provided by cool white fluorescent lamps external to the water bath. Light intensity was measured at the center of an empty column with a quantum meter (Lambda L1-185).

2.2. Experimental conditions

The initial semi-continuous cultures were regularly supplemented with nutrients by diluting the cultures with fresh modified mBG-11 from 35 to 40 to 10 μg Chl mL^{-1} (equivalent to 0.5 mg DW mL^{-1}) on every other day to maintain nutrient sufficiency and balanced growth. Cells were collected by centrifugation (1200 × *g* for 5 min), washed twice with double distilled water (DDW) and resuspended in the N-free mBG11 medium. The WT and P127 cultures were grown in N-free mBG11 medium with two initial Chl concentrations (designated ICC): 15 and 30 mg L^{-1} (biomass density of 0.5 and 1 mg mL^{-1}, respectively) under two light intensities of 75 (low light; LL) and 175 (high light; HL) μmol photons m^{-2} s^{-1}. Since *L. incisa* forms cell clusters, growth was monitored on the basis of Chl (mg L^{-1}) and DW (mg mL^{-1}) content as previously described [6].

2.3. Experiments with ROS-inducing chemicals

The *L. incisa* (WT and P127) cultures were grown in N-free mBG-11 media with ICC of 20 mg L^{-1} (0.7 mg DW mL^{-1}). The cultures (100 mL) were agitated in 250 mL Erlenmeyer flasks in an incubator-shaker at a speed of 170 rpm, at 25 °C and illumination of 50–60 μmol photons m^{-2} s^{-1}. Different concentrations of Rose bengal (RB) (Sigma-Aldrich, Steinheim, Germany), Methyl viologen (MeV) (Sigma-Aldrich, MO, USA) and hydrogen peroxide (H_2O_2) were added to the cultures after two days of N-starvation to ensure induction of LC-PUFA production. To facilitate penetration of the chemicals into the cells, cultures were gently sonicated for 2 min in a water bath sonicator (Transsonic T460, Germany) after addition of stock solutions in dimethyl sulfoxide (DMSO).

2.4. Measurement of photosynthetic parameters

The ratio of the variable to maximal fluorescence (F_v/F_m) is considered as a measure of the maximal quantum efficiency of PSII photochemistry [34]. F_v/F_m was determined on triplicate dark-adapted (5 min) algal samples (adjusted to 5.0 mg Chl L^{-1}) using an induction fluorometer plant efficiency analyser (PEA, Hansatech, UK), equipped with a liquid sample holder.

2.5. Lipid extraction and separation

To extract lipids, *L. incisa* culture samples were collected in 50 mL falcon centrifuge tubes and spun down at 1200 × *g*; the pellet was frozen at − 20 °C and lyophilized (10 Mr-TR, Virtis Co.). Lyophilized