Algal Research 27 (2017) 89–98

Contents lists available at ScienceDirect

Algal Research

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

The comparative study for physiological and biochemical mechanisms of *Thalassiosira pseudonana* and *Chaetoceros calcitrans* in response to different light intensities

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ARTICLE INFO

Keywords:
T. pseudonana
C. calcitrans
Light intensity
Physiological and biochemical responses

ABSTRACT

Both *Thalassiosira pseudonana* and *Chaetoceros calcitrans* are important species of marine microalgae. Their adaptive capacity to light intensity significantly varies during actual cultivation. We investigated the responses of these two species to different light intensities at 25 °C through analyses of photoacclimation, antioxidant ability as well as many physiological and biochemical parameters. The growth rate of *T. pseudonana* was positively correlated with light intensity in the first 8 days. However, after that, the greatest growth rate of *T. pseudonana* was found at a light intensity of 40 μmol $m^{-2} s^{-1}$. For *C. calcitrans*, the growth rate at 200 μmol $m^{-2} s^{-1}$ was the highest among the three light intensities in the first 10 days, while the growth rate at 120 μmol $m^{-2} s^{-1}$ became the highest after 10 days. Both algae could yield the most chlorophyll a at 40 μmol $m^{-2} s^{-1}$, and β-carotene content was increased in *T. pseudonana* but decreased in *C. calcitrans* with the increase of light intensity. Moreover, maximal photochemical efficiency of PSII (Fv/Fm) and effective photochemical efficiency of PSII (Fv′/Fm′) in *T. pseudonana* were decreased with the increase of light intensity, while non-photochemical quenching (NPQ) and superoxide dismutase (SOD) activities were increased. However, Fv/Fm and Fv′/Fm′ in *C. calcitrans* under all light intensities remained relatively constant, and Fv/Fm was maintained at around 0.6. NPQ was increased and SOD activity was decreased in *C. calcitrans* with the increase of light intensity. These findings suggested that *T. pseudonana* employed a more positive physiological strategy for adaptation to environmental stress under low light intensity, while *C. calcitrans* possessed a good physiological strategy for adaptation to environmental stress under high light intensity. Taken together, we provided a better understanding of the growth effects of these two microalgae under different light intensities and offered novel insights into their mass culture.

1. Introduction

As a type of phytoplankton with high nutrition value, microalgae are widely distributed in land and sea, and they have been used in many aspects of human life for years. Moreover, microalgae can produce a wide range of metabolites, such as protein, lipid, carbohydrate, carotenoid (Car) and vitamin, which can be used for aquaculture feeds, biofuels, cosmetics and energy production [1,2]. *Thalassiosira pseudonana* and *Chaetoceros calcitrans* are both important microalgae. For example, they have served as the aquaculture feeds of shellfish and crustaceans during their larval stage [3,4]. Moreover, *C. calcitrans* has been considered as a potential source of lipids for biofuels due to its high contents of lipids [5]. These two algae play a vital role in nutrient recycling and climate regulation [6]. For example, it has been widely realized that microalgae have great potentials for the production of valuable substances since they can more effectively utilize solar energy compared with higher plants. Microalgal growth and metabolism are affected by many environmental factors, such as light intensity, photoperiod, temperature and so on. Therefore, it is necessary to employ *T. pseudonana* and *C. calcitrans* as the model system to understand how to

Abbreviations: Fv/Fm, maximal photochemical efficiency of PSII; Fv′/Fm′, effective photochemical efficiency of PSII; rETR, relative electron transport rate; PFD, the intensity of actinic light; RLC, rapid light-response curves; NPQ, non-photochemical quenching; SOD, superoxide dismutase; Chl a, chlorophyll a; β-Car, β-carotene; Car, carotenoid; ROS, reactive oxygen species; SRM, selected reaction monitoring

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http://dx.doi.org/10.1016/j.algal.2017.08.026
Received 9 April 2017; Received in revised form 17 August 2017; Accepted 17 August 2017

optimize environment factors to improve microalgal growth and achieve higher yields of microalgae-derived by-products.

Light has been recognized as a key environmental factor for microalgal growth. However, excessive exposure to light can lead to the photooxidative damage, resulting in reduced growth of microalgae. Furthermore, the metabolic status of cells can be affected by light through altering the costs of carbon production and its allocation [7,8]. Microalgae have evolved various adaptive mechanisms in response to dynamic light environment [9]. As previously described, as one of the main natural factors, excessive light exposure can lead to the formation of reactive oxygen species (ROS) and the enhancement of antioxidant activity [10,11]. The defense mechanisms can protect microalgae from high light damage and oxidant stress since some biochemical energy generated by photosynthesis is diverted for defense against ROS and respiration, thereby strongly affecting the microalgal growth [12,13]. ROS-triggered photooxidative damage can be minimized by photoprotective mechanisms in the photosystem (PS) [14].

ROS, including superoxide anion ($O\cdot_2^-$), hydrogen peroxide (H_2O_2), single oxygen (O_2 ($^1\Delta_g$)) and hydroxyl radical (HO·), are normal by-products of oxidative metabolism in aerobic organisms but frequently stimulated by stress such as light stress. Under non-stressful conditions, the balance in ROS level is maintained by protective antioxidant mechanisms. However, such a balance is altered when exposed to stresses caused by various factors (e.g., temperature or high salt level), leading to a series of changes in metabolic processes related to stress acclimation, development or apoptosis [15]. The steady-state level of $O\cdot_2^-$ controlled by superoxide dismutase (SOD) is an important protective mechanism, since $O\cdot_2^-$ can act as highly reactive oxygen derivatives [16]. SOD is usually considered as the primary substance against oxidative damage, and SOD activity is increased with the enhancement of oxidative stress [17]. Pigments play a crucial role in microalgal growth as their levels reflect the microalgal potential to capture and convert light energy into biochemical energy [18]. Chlorophyll a (Chl a) has been closely correlated with photosynthetic rate, which is positively correlated with Chl a content [19]. Car can protect photosynthetic apparatus by quenching (O_2 ($^1\Delta_g$)), and β-carotene (β-Car) is a major form of Car [20]. The effects of light spectrum on pigment production in *Chlorella vulgaris* and *cyanobacteria Gloeothece* and high-light stress on pigment metabolism in *Parietochloris incise* have been well documented [21,22]. Analysis of chlorophyll fluorescence is a powerful tool to predict the physiological status of photoautotrophic organisms [23,24], which is highly sensitive, non-destructive and reliable for studying changes in photosynthetic organisms and its photosynthetic efficiency under different environmental conditions [25–27]. Several parameters have been reported to correlate with chlorophyll fluorescence. For example, non-photochemical quenching (NPQ) is related to non-photochemical dissipation of the absorbed light; Fv/Fm is the maximal photochemical efficiency of PSII; and Fv′/Fm′ is the effective photochemical efficiency of PSII. Fv/Fm is usually used as a tool for stress assessments in plant photosynthesis, and its value is relatively constant in non-stressed culture and decreased in stressed culture [28,29]. NPQ is increased with the decrease of PSII-related fluorescence emission [30], and it is an important photoprotective mechanism against photoinhibition, which can dissipate excess energy as heat [31]. Therefore, the determination of these indicators can better reveal the effects of light intensity on the growth and metabolism of microalgae.

Previous studies have examined the effects of temperature on growth, composition and nitrogen metabolism of *T. pseudonana* [32,33], as well as the effects of salinity, nitrogen and light on growth and optimal properties of *T. pseudonana* [34,35]. In addition, the effects of temperature, salinity and carbon dioxide on the growth and biochemical composition of *C. calcitrans* have been also documented [36,37]. However, it remains unclear how different light intensities affect the physiological and biochemical mechanisms of *T. pseudonana* and *C. calcitrans*.

In this study, we aimed to assess the growth and metabolic status of *T. pseudonana* and *C. calcitrans* under different light intensities. We examined the effects of different light intensities on the growth and physiological/biochemical mechanisms of *T. pseudonana* and *C. calcitrans* using above-mentioned indicators.

2. Materials and methods

2.1. Culture of microalgae

Marine diatoms *T. pseudonana* and *C. calcitrans* were obtained from the Marine Biotechnology Laboratory of Ningbo University (Zhejiang, China) and cultivated in No. 3 medium (pH 8.5, salinity 24‰) supplemented with 20 mg/L Na_2SiO_3 [38].The culture was maintained at 25 °C in three temperature-controlled illumination incubators equipped with white light emitting diodes allowing the monitoring and regulation of light intensity. The three illumination incubators were set at 200 μmol m^{-2} s^{-1}, 120 μmol m^{-2} s^{-1} and 40 μmol m^{-2} s^{-1}, respectively, with a 12:12-h light/dark cycle, and other conditions remained the same. Cell density was monitored under a microscope with a Hausser hemacytometer every 2 days until the end of the experiment. Growth rate (μ) was estimated according to the formula: $\mu = \ln(N_2 / N_1) / (t_2 - t_1)$, where N_2 and N_1 were cell concentrations at time points t_2 and t_1 respectively. The algae were harvested by centrifugation at 12,000 rpm (4 °C) for 10 min, then freeze-dried and stored at − 80 °C for the measurement of photosynthetic pigments.

2.2. Pigment extraction and determination

Pigments were extracted and determined as previously described [39]. About 5 mg of freeze-dried samples of *T. pseudonana* and *C. calcitrans* were added into 2-mL centrifuge tubes containing 0.9 mL cold acetone. The mixture was placed in an ice-water bath and sonicated for 2 min. Subsequently, 0.1 mL ultrapure water was added to the centrifuge tubes, making a final content of acetone 90%. The centrifuge tubes were then stored in the refrigerator at − 20 °C overnight to extract pigments. Pigments were detected by HPLC-QqQ-MS. Before the detection, samples in centrifuge tubes were centrifuged (12,000 rpm) for 10 min, impurities and cell debris were filtered from supernatant by PTFE filter membranes, and then 0.5 M ammonium acetate solution was added to improve the degree of separation and peak shape. Processed samples were detected in 48 h to minimize the impacts of pigment degradation on the final results. Pigments were separated by TSQ Quantum Access HPLC system using Supelco Discovery C_{16} amino chromatographic column (150 mm × 4.6 mm, 5 μm, maintained at 30 °C). Elution was carried out with the mobile phase containing methyl alcohol (A), acetonitrile (B) and 0.5 M ammonium acetate solution (C) at a flow rate of 1.0 mL/min using the gradient as follows: 0 min, 80% A, 20% C; 7 min, 72% B, 20% C; 11 min, 77% B, 18% C; 19 min, 85% B, 2% C; 30 min, 80% B, 20% A; 34 min, 60% B, 40% A; 36–40 min, 80% A, 20% C. The mass spectrometer was operated in the positive-ion mode with selected reaction monitoring (SRM) under the following conditions: spray voltage, 2.5 kV; vaporizer temperature, 300 °C; flow rate of sheath gas pressure (N_2), 20 L/min; flow rate of auxiliary gas pressure (N_2), 5 Abs; and capillary temperature, 320 °C. Pigments were identified based on their retention time and absorption spectra as well comparison with authentic standards, which were purchased from Sigma-Aldrich (St. Louis, MO, USA). Data acquisition and analysis were performed with Xcalibur (Thermo Fisher Scientific).

2.3. Determination of SOD activity

Cells of *T. pseudonana* and *C. calcitrans* were harvested by centrifugation at 12,000 rpm for 10 min, washed with 0.05 M potassium phosphate buffer (pH 7.8) and resuspended in the same buffer. Cells were homogenized by sonication using a Cole Parmer CP600 4710 Ultrasonic Homogenizer. The cell homogenate was centrifuged at