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Strong-light-induced yellowing of green microalgae *Chlorella*: A study on molecular mechanisms of the acclimation response

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ABSTRACT

Two strains of unicellular microalgae, *Chlorella prototheocoides* and *Chlorella vulgaris*, subjected to strong light conditions (400 μmol photons m^{-2} s^{-1}) turn yellow, as compared to the green control cells cultured at low light intensity (80 μmol photons m^{-2} s^{-1}). Such a mechanism is typically interpreted in terms of an adaptive response of algae to overexcitation conditions. In the present work, fluorescence spectroscopy and molecular imaging techniques: fluorescence lifetime imaging microscopy and Raman imaging microscopy, were applied to readdress the problem: whether this process is associated with an acclimation or rather is a manifestation of a photo-degradation process. Yellow coloration of the algal culture exposed to strong light, was found to be associated with accumulation of xanthophyll pigments, predominantly zeaxanthin. The results show that carotenoids, newly synthesized in response to strong light conditions are not energetically coupled to chlorophylls and therefore are not photosynthetically active. On the other hand, over-synthesized xanthophylls can be potentially active as antioxidants, membrane stabilizing agents and, importantly, in shielding cells from intensive radiation, via "molecular sunglasses" mechanism. The latter mechanism has been identified in a cell nucleus and concluded to protect a genetic material against photodamage. Culturing *Chlorella* at elevated light intensities may also be considered as an alternative source of zeaxanthin, one of the macular pigments protecting human eyes against the age related macular degeneration.

1. Introduction

The growing interest in use of unicellular algae as alternative sources that can replace fossil fuels is associated with their biotechnological potential. Microalgae have an ability to produce valuable intracellular compounds, e.g., triacylglycerol, carotenoids, carbohydrates and vitamins, which are of interest for production of biofuels, dyes, food and feed [1]. Carbon dioxide sequestration, occurring during biomass production in the process of photosynthesis, is one of the main advantages that favour using algae as alternative renewable sources. Another advantage of microalgae is the possibility of using non-arable or degraded land for cultivation thereof and the fact that due to their fast growth rate they require a smaller land area than traditional agricultural crops to produce similar yields [2].

As photosynthesizing organisms, microalgae require light as an energy source, besides CO_2, water, inorganic nutrients, mainly nitrogen, and phosphate, in order to grow [3,4]. To develop the technology of the production of biofuels from microalgae, it is necessary to determine in detail the impact of each factor on the productivity of these organisms [5]. Light intensity is one of the main environmental factors influencing photosynthetic efficiency, growth, and cellular metabolism of microalgae. In outdoor cultivation, microalgae are exposed to diurnal and seasonal changes in light intensity; the movement of algae in large pools is also associated with strong light gradients according to the tank depth and culture density. Similar to higher plants, microalgae have evolved mechanisms of cellular and molecular response to optimize light harvesting [6]. The photoacclimation process includes regulation of the number of functional pigment-protein complexes of photosystems and amount of pigments associated with them, depending on light intensity [3,7]. Although indispensable for biochemical processes, light can have a negative effect on microalgae. Excess illumination can cause damage to algal cells through various processes, such as formation of reactive oxygen species in the thylakoid membranes [5]. Photosynthetic microalgae have evolved several strategies to reduce photodamage, among others via dissipation of excess light energy as heat through the so-called nonphotochemical excitation quenching (NPQ) and possibly via state transitions [8]. Mechanisms that prevent photodamage cause reduction of light use efficiency and are energy demanding, which is reflected in a decrease in the rate of microalgae growth and productivity [9,10]. Sustenance of biomass productivity in a broad range of light intensity requires knowledge of processes responsible for the decline in the effectiveness of

Abbreviations: Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; FLIM, fluorescence lifetime imaging microscopy; PS II, photosystem II; NPQ, nonphotochemical quenching.

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light utilization under strong illumination [11]. One of the mechanisms observed in different microalgae exposed to strong light conditions is the additional synthesis of carotenoids [12–17]. This particular response, in two strains of unicellular green microalgae, *Chlorella protothecoides* and *Chlorella vulgaris*, is analysed in the present work with application of fluorescence spectroscopy and two imaging techniques: fluorescence lifetime imaging microscopy and Raman imaging microscopy.

2. Materials and methods

2.1. Microalgal strains and medium

Strains of unicellular green microalgae *C. protothecoides* (SAG 211-7b) and *C. vulgaris* Beijerinck (CCLA 267) were obtained from the SAG Culture Collection of Algae and Culture Collection of Autotrophic Organisms. *C. protothecoides* was cultivated in Polytoma medium (according to Medium Recipe SAG) and *C. vulgaris* was cultivated in Bold's Basal Medium. The medium was sterilized in an autoclave before inoculation. The inocula were maintained in sterile media mentioned above in shaken Erlenmeyer flasks under conditions: 80 μmol photons m^{-2} s^{-1} of continuous light and at a constant temperature of 24 ± 1 °C.

2.2. Growth analysis

Growth experiments were carried out in 150 ml sterilized medium in 300-ml Erlenmeyer flasks. The cultures were grown under continuous light intensity: 400 μmol photons m^{-2} s^{-1} (referred to as a high light intensity, HL) and 80 μmol photons m^{-2} s^{-1} (low light intensity, LL) of photosynthetically active light provided by a fluorescent lamp with orbital shaking at 100 rpm. The growth temperature was 24 ± 1 °C. The cultures were grown in batch culture for 9 days.

Algal growth was determined by assessment of daily changes in optical density OD_{650} for 9 days using a Cary 300/Biomelt spectrophotometer. The specific growth rate μ (day^{-1}) in the logarithmic growth phase was calculated by:

$$\mu = \ln(N_2/N_1)/(t_2-t_1) \quad (1)$$

where N_1 is the initial biomass concentration at time t_1 and N_2 is the biomass concentration at time t_2.

The experiments were carried out in three replications. Differences in the measured parameters were compared using one way ANOVA followed by a Tukey HSD test (*STATISTICA 10*).

2.3. Modulated chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were performed using a DUAL-PAM 100 Chlorophyll Fluorometer (Heinz Walz Gmbh, Effeltrich, Germany) in 10 ml quartz glass cuvette. Homogeneous distribution of algae cells in the cuvette was maintained by a sample mixing with the use of a magnetic micro stirrer. Prior to measurements, samples were dark adapted for 30 min, at 24 °C.

Algae samples were subjected to saturation pulse analysis at red (620 nm) actinic light, intensity 45 μmol photons m^{-2} s^{-1} or 224 μmol photons m^{-2} s^{-1} for 12 min. A Dual PAM software (v 1.9) was used to record all data.

Maximum quantum efficiency of Photosystem II was calculated according to the formula:

$$F_v/F_m = (F_m-F_o)/F_m \quad (2)$$

where F_v, F_m and F_o refer to variable fluorescence, maximum fluorescence and fluorescence level recorded at very low light intensity, when all reaction centres are open, respectively [18]. The dimensionless coefficient nonphotochemical quenching (NPQ), an "indicator" for excess excitation energy dissipated as heat, that cannot be used in CO_2 fixation, was calculated according to the general formula:

$$NPQ = (F_m-F_{m\prime})/F_{m\prime} \quad (3)$$

Coefficient of photochemical fluorescence quenching being a measure of the fraction of open PSII reaction centres:

$$q_P = (F_{m\prime}-F)/(F_{m\prime}-F_{o\prime}) \quad (4)$$

where $F_{m'}$ and $F_{o'}$ refer to maximum fluorescence level determined during actinic irradiation and fluorescence level determined directly after turning off the actinic irradiation, respectively.

2.4. Pigment analysis

Concentration of chlorophylls and carotenoids was determined in 80% acetone as described elsewhere [19]. Composition of the carotenoid pool was analysed chromatographically by HPLC technique (on a phase-reversed, C-18 column) with Shimadzu (Japan) LC-20AD system. A solvent system acetonitrile:methanol:water (72:8:3,v:v:v) was used as a mobile phase in the initial phase of pigment separation and it was changed to methanol:hexane (4:1, v:v) after elution of zeaxanthin. Flow rate was stabilized at 0.8 mL min^{-1}. UV–Vis absorption spectra

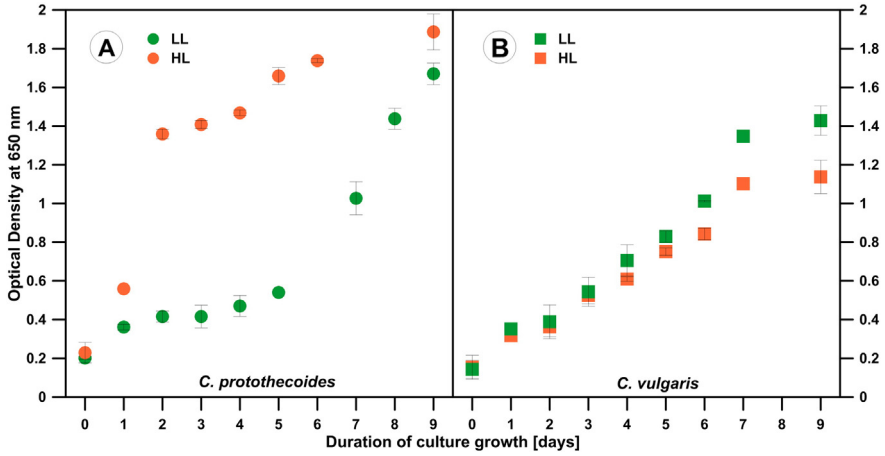

Fig. 1. Comparison of growth of *C. protothecoides* (A) and *C. vulgaris* (B) under low light intensity (LL) and high light intensity (HL). Growth rate is expressed in optical density (pathlength 1 cm) at 650 nm. Data are arithmetic means (±S.D.) of 9 measurements.