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Light-mediated lutein enrichment of an acid environment microalga

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ABSTRACT

Algae fully acclimated to different light intensities express different characteristics. At low light intensities, most algae produce more light-harvesting pigments to improve their photosynthetic efficiency. In contrast, at high light intensities, some algae produce high concentrations of "sunscreen" pigments to protect the cell from exposure to excess ultraviolet and PAR light. *Coccomyxa onubensis* grows selectively at pH 2.5, which is a competitive advantage for massive production. The alga pigment profile is rich in carotenoids, especially lutein. In this research we studied the effect of low to moderate light intensity shifts on lutein accumulation of *C. onubensis* cultures, doubly aimed at understanding the light-dependent role of main carotenoids in acid-tolerant microalgae and at developing strategies to induce β-carotene and mainly lutein accumulation with applied purposes. *Coccomyxa* cells were grown at 50, 140 and 400 μmol photons m^{-2} s^{-1}, reaching their maximum growth rates and carotenoid productivities at 400 μmol photons m^{-2} s^{-1}. Lutein accumulation slightly depended on biomass concentration and maximum productivities of biomass and lutein were achieved in relatively dense cultures of 0.7 and 1 g L^{-1}. The main results indicate that *C. onubensis* is a very promising lutein accumulating microorganism if incubated under a suitable cultivation strategy mainly consisting of transferring relatively low biomass concentration cultures either from low to moderate light intensity or from moderate to low light intensity, therefore profiting from either pigment light-capturing or light-dissipation activities.

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1. Introduction

Light is one of the major factor influencing algal growth, which has been confirmed through studies of the occurring relationships between light intensity and algal density, chlorophyll concentration, and photosynthesis activity [1,2]. At low intensities, light can become growth-limiting as the mutual shading of cells causes steep gradients of light. Photoacclimation mainly consists of biochemical and morphological changes in the algal cells in order to produce a more efficient light use of the incident light in the cultures or to protect the photosynthetic machinery from excess light. Under low light intensities, algae photoacclimate by increasing their cellular chlorophyll contents at low intensities, through changes in the numbers of photosynthetic unit (PSU) [3] and/or in PSU size [4,5]. By contrast, when algae are exposed to excess light intensities with respect to those required to saturate photosynthesis, the excess light becomes a stress factor, and can cause photo-damage. Powles [6] suggests that photo-damage will occur when there is an imbalance between light energy absorption and utilization in photosystem II (PSII). Algae have developed mechanisms to adapt to excess light [7–9]. Some mechanisms consist of dissipation of the excess photons absorbed thorough the light-harvesting antenna complexes of PSII [10]. If those relaxation mechanisms are unable to dissipate all of the excess energy, the remaining flux of excess photons leads to the formation of harmful radicals.

The structure and properties of carotenoids are in relation to their main functions. In addition to the provitamin A activity of some carotenoids (such as β-carotene), they also have other functions such as light-harvesting pigments or as antioxidants protecting cells against the harmful effects of reactive oxygen species (ROS) [11–14]. Some carotenoid pigments may provide effective protection against disadvantageous influence of light [15,16] by dissipation of light energy through non-photochemical processes. Carotenoids are present in the photosynthetic antenna complexes of plants and algae. These complexes capture energy at their characteristic wavelengths and transfer it to chlorophylls, expanding the spectrum of light that an organism can use for photosynthesis. Most of carotenoids are xanthophylls (such as lutein and astaxanthin). Carotene consists of hydrocarbon chain, composed solely of carbon (C) and hydrogen (H), which constitute less than 10% of all carotenoid species as β-carotene. Light-harvesting complex II (LHCII) antenna complex described the existence of xanthophyll binding sites. Molecules of lutein and neoxanthin take part in light capture

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and dissipation of light energy. Pigments of the so-called xanthophyll cycle also play a role in light capture – violaxanthin – and in photoprotection through dissipation of excess energy — zeaxanthin. Moreover, several carotenoids and xanthophylls are also implicated in detoxification of reactive forms of oxygen that are formed during photosynthesis. In higher plants, Croce et al. [17] state that two of three binding sites (L1 and L2) possess the highest affinity to lutein, this pigment being the major carotenoid of *Coccomyxa onubensis* (more than 75% of total carotenoid pool).

So far, no single study exists which adequately covers how light intensity affects *Coccomyxa* growth and the role of carotenoids. Interestingly, some previous studies in our laboratory showed *C. onubensis* to have certain sensitivity to high light intensity (summer irradiances), resulting in low growth [18]. Acidophilic microalgae have been found to grow much slower than the so-called "common" microalgae [19, 20]. However, we have already demonstrated that low pH medium and suitable growing conditions [21], excluding high light intensities, promote growth, therefore making acidophilic microalgae biomass production an attractive choice for outdoor cultivation systems, with the added advantage of having quite a selective low pH culture medium making open culture competitors extremely scarce.

Therefore, obtaining fast growth and suitable conditions to promote lutein accumulation at the same time was the predominant challenge in the research regarding acid-tolerant microalga, aimed at proving its value for lutein-rich biomass production processes. Regarding lutein accumulation, some chemical parameters (nutrients, meta-ions) have already been shown to enhance its biosynthesis [18,21,23]. The aim of this manuscript is to show that lutein biosynthesis might be particularly active when *C. onubensis* is cultivated with transits in light intensity, either from low light to moderate or moderate to low light. In this research, we compared steady-state exposures to low light with higher light exposures. Investigations have been focused on various aspects of the differences between acclimated and non-acclimated cell cultures when light shifts from low to moderate intensities and vice versa. Algal growth rate, biomass productivities and variation on chlorophyll and carotenoid contents were studied. Particularly interesting in this study is lutein content, a major pigment of *C. onubensis*, since lutein has gathered increasing attention on the grounds of recent studies that show how an adequate intake of this product by humans might help prevent or ameliorate the effects of degenerative human diseases among others [27].

Several microalgae have been proposed as potentially adequate lutein sources, such as *Muriellopsis* sp., *Chlorella zofingiensis*, *Chlorella protothecoides* and *Scenedesmus almeriensis* [24–27]. *C. onubensis* accumulates 5–6 mg g^{-1} dry weight, which is within the upper range of lutein concentrations accumulated by the mentioned microalgae. However, compared to continuous cultivation of other lutein producing species, *C. onubensis* has the practical advantage of growing well in an extremely selective culture medium at very low pH which preserves cultures from microbial contamination [18]. This gives the acid-tolerant microalga an attractive potential as a producer of this photosynthetic pigment.

2. Materials and methods

2.1. Isolation

C. onubensis was isolated from acidic waters of the Tinto River (Huelva, Spain). This river has some very special features, such as low pH and a high concentration of heavy metals, especially iron, copper, magnesium and aluminum. An axenic culture of the algae was obtained by streaking it on basal agar medium at pH 2.5, which was then transferred to the liquid medium. *Coccomyxa* has been recently identified by ribosomal 18S subunit rDNA sequence analysis. The identified 18S subunit rDNA sequence was registered at GenBank with accession number GU265559.

2.2. Culture conditions

According to the chemical composition of the natural environment, cultures were grown at pH 2.5 in a culture medium based on K9 medium [28]. A modified K9 medium was prepared according to the following composition: 3.95 g K_2SO_4, 0.1 g KCl, 0.5 g K_2HPO_4, 0.41 g $MgCl_2$, 2.29 g KNO_3, 0.01 g $CaCl_2$, and 5 mL Hutner solution [29]. For different experiments, the cultures were inoculated from a repeated batch culture of *C. onubensis* and then adapted to the mode of cultivation required for each specific experiment. The mode of cultivation for each experiment is described throughout the Results and discussion section. The cultures were cultivated in one-liter roux bottles under constant illumination provided by fluorescent lamps at three intensities: 50, 140, and 400 (μmols m^{-2} s^{-1}) PAR and with constant bubbling with CO_2: air mixture (5:95, v/v) at 27 °C. The bubbled gas flow rate was 190 mL min^{-1} per liter.

2.3. Dry weight measurements and growth rate calculations

To measure dry weight, 10 mL samples of each culture were used. The samples were passed through Whatman glass microfiber filters of 47 mm in diameter and 0.7 μm pore size using a vacuum pump to separate the cells from the medium. The filters with the cells were then dried in a stove at 90 °C during 24 h.

Dry weight data were used to calculate growth rates. Specific growth rates of cultures were calculated using the following expression:

$$\mu = \mathrm{Ln}(C/C_0)/t,$$

where μ is the specific growth rate, C_0 is the initial biomass concentration (dry weight), and C is the biomass concentration at any time t.

In adapted semi-continuous cultures average growth rates were calculated as the average daily growth using dry weight data. When cultures were light shifted, specific growth rates were calculated during the subsequent 24 h.

2.4. Quantum yield

Fluorescence measurements were made as the maximum quantum yield (QY) of PSII (F_v/F_m). It was measured to evaluate the viability of the cells. It was determined using pulse amplitude modulation (PAM 210, Walz, Germany) [30]. Samples of each culture were previously adapted to darkness for 15 min.

2.5. Chlorophyll and carotenoid determination

Carotenoids were extracted using aliquots (1 mL) of the cultures. Cells were spun down for 8 min at 13,000 ×g. The obtained pellet was placed in 60 °C water for 5 min. The pellet was resuspended in 1 mL of methanol and the suspension was shaken vigorously for 1 min and centrifugated for 8 min at 3000 ×g. Carotenoids and chlorophylls were separated and identified by HPLC (ThermoQuest, Thermo Separation products) with a RP-18 column, using a modified method described by Young [31]. In the mobile phase, solvent A was ethyl-acetate and solvent B was acetonitrile and water (9:1, v/v). External standards (DHI, http://c14.dhigroup.com/) and their corresponding calibration curves were used to identify and quantify both lutein and β-carotene.

2.6. Statistics

Unless otherwise indicated, tables and figures show means and standard deviations of three independent experiments.

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