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Effect of UV radiation or titanium dioxide on polyphenol and lipid contents of *Arthrospira* (*Spirulina*) *platensis*

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ABSTRACT

Phenolics and lipids of *Arthrospira* (*Spirulina*) *platensis* are important compounds with attractive properties. The influence of two oxidative stress conditions (UV irradiation and TiO_2 concentration) on the production of these added-value compounds was investigated. UV and TiO_2 stresses were shown not to influence the final concentration of biomass that resulted to be 4.83 and 5.08 g L^{-1}, respectively. Compared to the control run, total polyphenol yield decreased by 7.5, 24.1 and 23.1% after UV exposure for 3, 6 and 9 h, respectively, whereas in the presence of 0.10, 0.25 and 0.50 g L^{-1} of TiO_2 such a yield increased by 8.4, 7.0 and 7.3%, respectively. However, the treatment with 9 h of UV stress led to a lipid content increase of 29.5%, but with a reduction of the FAME fraction (67.3%). While the TiO_2 stress lead to a substantial decrease of total lipid fraction. These results on the whole suggest that UV irradiation could be usefully exploited to increase the lipid content of microalgae or cyanobacteria for different applications in bioenergy production (e.g. combustion, gasification and pyrolysis), while the increased phenolic content induced by the oxidative stress with TiO_2 may be of some interest in both the pharmaceutical and cosmetic sectors.

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1. Introduction

Cyanobacteria are one of the most ancient forms of photosynthetic prokaryotes that show a considerable potential not only for bioenergy production but also for high added-value foods and pharmaceutical products [1]. They are in fact recognized as sources of novel biologically active compounds with protective effects against viral, bacterial and fungal infections, cancer, allergy, hyperlipidemia and inflammation [2–5]. Among cyanobacteria, *Arthrospira* (*Spirulina*) *platensis* plays a fundamental role because of its high nutritional value related to its content of a number of essential nutrients such as vitamins, minerals, proteins and polyunsaturated fatty acids (PUFAs) [6].

A. platensis can easily be recognized by the main morphological feature of its genus, i.e. the arrangement of multicellular cylindrical trichomes in a helix along the entire length of filaments. For centuries, native people from Chad Lake in Africa and Texcoco Lake in Mexico have used it as an important source of food, because of considerable nutraceutical properties [7]. Nowadays, this cyanobacterium has attracted attention for its capability to produce antioxidant compounds such as phenolics [8]. These compounds play a key role against chronic-degenerative diseases and aging induced by free radicals, in particular the reactive oxygen (ROS) and nitrogen (RNS) species.

Radicals come from different processes, among which are endogenous enzymatic reactions, water hydrolysis caused by UV irradiation and TiO_2-dependent photocatalysis. UV radiations are very dangerous because they lead to an increase in the ROS level and, consequently, to negative effects such as lipid peroxidation and DNA strand breakages [9].

TiO_2 photocatalytic activity depends on titanium dioxide concentration and TiO_2 crystal structure [10]. Its nanoparticles were shown to cause toxicity to organisms, by producing ROS such as the hydroxyl radical (·OH) and the superoxide anion ($\cdot O_2^-$) upon interaction with light, hence leading to cell membrane damages [11].

A. platensis is a well-known source of dietary polyunsaturated fatty acids, one of the most attractive of which is γ-linolenic acid (GLA) that has been studied for its ability to decrease blood cholesterol levels, treat atopic eczema and alleviate the symptoms of premenstrual syndrome [12,13]. The culturing of such cyanobacterium is relatively inexpensive and simple, since it can grow quickly, is resistant to unfavorable environmental conditions and can efficiently be harvested [14]. Taking into account that growth conditions and nutrient availability can strictly modulate chemicals production, special strategies have to be adopted to cultivate *A. platensis* with the aim to increase the productivity of biomass especially rich in a desired compound [15]. Recent works suggest that its polyphenol content and lipid fraction can be increased by acting on parameters such as light intensity, nitrogen and sodium chloride concentrations [16,17] and temperature [18].

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Based on this background, the aim of this work is to evaluate whether, and eventually, how intra- and extracellular polyphenol and lipid production by this cyanobacterium can depend on the time of exposure to UV radiation or the TiO_2 concentration.

2. Materials and methods

2.1. Chemicals

Medium salts, chloroform, methanol, hexane, Folin–Ciocalteu reagent, sodium carbonate, titanium isopropoxide, 2-propanol and GC standards (methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl α-linolenate and methyl γ-linolenate) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methyl esters standard stock solution was prepared with hexane and stored at −20 °C until use. An aqueous solution of titanium isopropoxide and 2-propanol was mixed at room temperature (20 ± 1 °C) for 4 h to obtain a homogeneous gel of TiO_2 in anatase form, which was subsequently dried for 12 h at 100 °C.

2.2. Cultivations under oxidative stress conditions

A. (*Spirulina*) *platensis* was obtained from the Culture Collection of Algae of the University of Texas (UTEX). Cells were grown in duplicate at room temperature in the medium of Schlösser [19] in a 3.5 L-horizontal tubular photobioreactor exposed to 82 ± 5 μmol photons m^{-2} s^{-1} light intensity [20]. The pH was regulated at 9.5 ± 0.2 through the daily addition of CO_2. At the early stationary phase (8 days), biomass was collected as early control (0^A). For subsequent stress tests, 0.5 L-aliquots of cell suspension were harvested after the same time from the photobioreactor and transferred to 1.0 L-Erlenmeyer flasks. Biomass was exposed for 3 h per day during 1, 2 and 3 days to artificial UV radiation, generated by a 20 W-fluorescent lamp, model TUV T8 (Philips, Eindhoven, The Netherlands), or to anatase TiO_2 at concentrations of 0.10, 0.25 and 0.50 g L^{-1}. The 1.0 L-Erlenmeyer flasks were exposed to 82 ± 5 μmol photons m^{-2} s^{-1} light intensity, and biomass was kept in suspension by bubbling air. In order to better evidence these stress effects, additional control runs (0^B) were carried out using biomass harvested from the photobioreactor at the early stationary phase and grown into 1.0 L-Erlenmeyer flasks without UV or TiO_2 for five days. To provide biological replication, each stress condition and control runs were performed in duplicate. Biomass concentration (X) was determined daily by absorbance measurements at 560 nm (Abs_{560}) by an UV–visible spectrophotometer, model Lambda 25 (PerkinElmer, Milan, Italy). All measurements were done in triplicate, and X, expressed in milligrams of dried biomass per liter of medium (mg_{DB} L^{-1}), was related to absorbance by the equation ($R^2 = 0.9921$):

$$Abs_{560} = 0.0024X - 0.1129. \quad (1)$$

In order to remove any variability on biomass dry weight due to TiO_2 biosorption, the ash contents of samples either of the control (0^B) or of dry *A. platensis* biomass treated with TiO_2 (0.5 g L^{-1}) were determined at 750 °C for 4 h. The influence of TiO_2 powder biosorbed onto *A. platensis* (12.43 ± 0.99 $g_{ash}/100g_{DB}$) was statistically insignificant ($p < 0.05$) compared with the control (11.63 ± 0.93 $g_{ash}/100g_{DB}$).

At the end of these stress treatments, cells were observed with a magnification of 100× and images acquired by a DMLS optical microscope equipped with a DC 200 digital camera (Leica, Wetzlar, Germany). Then, they were separated from culture medium by centrifugation (centrifuge ALC 42426, Milan, Italy) for 15 min at 3500 rpm, dried at 105 °C for 24 h and pulverized in a mortar. The centrifuged medium was immediately frozen and stored at −20 °C for the later analysis.

2.3. Polyphenol and lipid extractions

In order to recovery the polyphenol fraction, dried biomass was extracted following the methodology described by Shanab et al. [21] with some modifications. Briefly, *A. platensis* dried powder was placed in 15 mL-amber glass test tubes with screw caps on a magnetic stirrer, model Mr. 2002 (Heidolph, Kelheim, Germany) for 19 h in the dark and at room temperature. The extraction was performed using distilled water as extractive solvent with a constant solid/liquid ratio of 1:10 [22]. After every extraction, the liquid was separated from cell debris by centrifugation (6000 × *g*) 10 min (ALC PK 131 Centrifuges, Alberta, Canada) and immediately frozen at −20 °C [23] before the analysis. All the extractions were performed in triplicate.

The lipid fraction of biomass was extracted following the method described by Krienitz and Wirth [24] with some modifications. Lipids were extracted with an ultrasonic bath, model UTA 90 (FALC, Treviglio, Italy), for 30 min combined with a 5 h-reflux extraction using a mixture of chloroform and methanol (2:1, *v*/*v*).

2.4. Determination of polyphenol and lipid contents

Intra- and extracellular phenolic compounds were quantified by the Folin–Ciocalteu assay [25]. For intracellular polyphenol analysis, 4.80 mL of distilled water, 0.20 mL of sample and 0.50 mL of Folin–Ciocalteu reagent were added to 15 mL test tubes. The content was mixed, and 1.00 mL of a 20% (*w*/*v*) sodium carbonate solution was added, followed by the addition of distilled water to reach the final volume of 10 mL [26]. For extracellular polyphenol analysis, 2.50 mL of cell-free medium, 0.05 mL of Folin–Ciocalteu reagent and 0.10 mL of 20% (*w*/*v*) sodium carbonate solution were mixed. All the solutions were allowed to stand at room temperature and in the dark for 1 h. The concentration of intracellular polyphenols in the aqueous extract (TP_i) and that of extracellular polyphenols in the medium (TP_e) were determined by absorbance measurements at 725 nm (Abs_{725}) using the same UV–visible spectrophotometer as above and expressed in milligrams of gallic acid equivalents per milliliter of solvent (mg_{GAE} mL^{-1}). Calibration curves of TP_i and TP_e were made with standard solutions of gallic acid and described by the equations:

$$Abs_{725} = 0.0017\,TP_i \quad \left(R^2 = 0.9966\right) \quad (2)$$

$$Abs_{725} = 0.0015\,TP_e \quad \left(R^2 = 0.9972\right). \quad (3)$$

2.5. Growth and yield parameters

The mean specific growth rate (μ), expressed in day^{-1}, was calculated by the equation:

$$\mu = \frac{1}{t}\ln\left(\frac{X_m}{X_0}\right) \quad (4)$$

where t (days) is the growth time, and X_m and X_0 (g_{DB} L^{-1}) are the biomass concentrations at the beginning of the stationary growth phase (8 days) and the beginning of cultivations, respectively.

The intracellular polyphenol yield (Y_i), expressed in mg_{GAE} g_{DB}^{-1}, was calculated as:

$$Y_i = \frac{TP_i \times V}{W_{DB}} \quad (5)$$

where V (mL) is the volume of solvent used for the extraction and W_{DB} (g_{DB}) the dried biomass mass taken either before (8 days) or after (13 days) the stress treatment.

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