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Streptomycin affects the growth and photochemical activity of the alga *Chlorella vulgaris*

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ABSTRACT

Antibiotics are increasingly being used in human and veterinary medicine, as well as pest control in agriculture. Recently, their emergence in the aquatic environment has become a global concern. The aim of this study was to evaluate the effect of streptomycin on growth and photosynthetic activity of *Chlorella vulgaris* after 72 h exposure. We found that growth, photosynthetic activity and the content of the D1 protein of photosystem II decreased. Analysis of chlorophyll *a* fluorescence emission shows a reduction in the energy transfer between the antenna complex and reaction center. Also the activity of the oxygen evolution complex and electron flow between Q_A and Q_B were significantly reduced; in contrast, we found an increase in the reduction rate of the acceptor side of photosystem I. The foregoing can be attributed to the inhibition of the synthesis of the D1 protein and perhaps other coded chloroplast proteins that are part of the electron transport chain which are essential for the transformation of solar energy in the photosystems. We conclude that micromolar concentrations of streptomycin can affect growth and photosynthetic activity of *Chlorella vulgaris*. The accumulation of antibiotics in the environment can become an ecological problem for primary producers in the aquatic environment.

1. Introduction

Occurrence of pharmaceuticals in the aquatic environment has become a matter of ecological concern. These substances are not routinely monitored because they are often not included in the environmental legislation and the environmental fate is poorly understood or not studied at all (Zenker et al., 2014). Some of these chemicals are poorly removed by the wastewater treatment plants and cannot be completely removed, resulting in the continuous discharge of toxicants to the aquatic environment (Gavrilescu et al., 2014; Yu et al., 2014). The main emission sources of pharmaceuticals antibiotics in urban areas are hospitals and wastewater treatment plants (Lin et al., 2008). Also a large portion of drugs in the water come from improper disposal of unused or unwanted medicines from households and medical facilities (Bound et al., 2006).

Antibiotics are among the most commonly prescribed drugs in the world; these molecules have become a new class of emerging environmental contaminants that are extensively and increasingly being used in human and veterinary medicine, as well as in aquaculture and agriculture (Kümmerer, 2009). Wise (2002) estimated that the worldwide antibiotic consumption varies from 100,000 and 200,000 t per year. In 1999, the total amount of antibiotics used in the European Union and Switzerland was 13,288 t of 8637 (Sukul and Spiteller, 2007). In veterinary medicine, more than 70% of ingested drugs are antibiotics (Halling-Sorensen et al., 1998). In addition, at the fish farms, antibiotics are added to foods to prevent infections in the fish, so in this way antibiotics are introduced to aquatic environment directly (Qian et al., 2012).

Many of the antibiotics used in human and veterinary treatment, are excreted unchanged in the urine and feces so remaining active (Kümmerer, 2009). The concern of the antibiotic release to the aquatic environment is the fact that these compounds may affect non-target organisms. This is because many aquatic organisms share molecular receptors and cellular processes (for example, protein synthesis) with pathogens (Kümmerer, 2009).

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Streptomycin is an aminoglycoside antibiotic originally isolated from *Streptomyces griseus*. Its action is on the ribosomes by interfering with the binding of aminoacyl-tRNA, causing an erroneous reading of the genetic code, inhibition of initiation of translation of mRNA and aberrant proofreading (Pelchovich et al., 2013). Streptomycin has been widely used for prophylactic and therapeutic purposes in human and veterinary medicine and as a phytosanitary agent (van Overbeek et al., 2002). In the United States streptomycin was registered as an antibiotic for human use, as well as bactericidal/bacteriostatic antibiotic to control bacterial and fungal diseases of selected fruit, vegetables, seeds, general crops, ornamental crops, and as an inhibitor of the growth of microalgae in ornamental ponds and aquariums (USEPA, 1992).

The toxic effect of streptomycin on photosynthetic organisms in aquatic environments lies in the fact that streptomycin binds to small subunit of the prokaryotic ribosomes, which are similar to those found in microalgae chloroplasts, causing a selective inhibition of protein synthesis in the chloroplast of the alga. The absence of protein synthesis in the microalgae chloroplast, particularly high turnover proteins involved in the absorption of light, the electron transport or fixing carbon dioxide, could cause a reduction of the accumulated biomass and therefore a reduction in growth rate. Microalgae are the first link in the trophic chain in aquatic environments, so that any change in the dynamics of growth of these organisms could cause changes in aquatic ecosystems at higher taxonomic levels (Qian et al., 2012).

The effect of this and other antibiotics that affect protein synthesis in the chloroplast, has been poorly studied from a metabolic point of view, so the aim of this work was to study the relationship between growth and photosynthetic activity *Chlorella vulgaris* growing in the presence of streptomycin.

2. Materials and methods

2.1. Alga culture

Experiments were conducted using axenic cultures of the microalgae *Chlorella vulgaris* (Chlorophyta, Chlorellales). Stock cultures were obtained from the Laboratorio de Biotecnología de Microalgas (CINVESTAV, Mexico). The cells were autotrophically grown in batch cultures (250 ml) using an EDTA (ethylenediaminetetraacetic acid) free medium (Perales-Vela et al., 2007).

The growth conditions were: 22 ± 3 °C with a photon flux density of 200 μmol m^{-2} s^{-1}, and a 14-h light/10-h dark cycle. The cultures were aerated with filtered (0.22 μm pore) air (200 ml/min) to provide a constant concentration of CO_2. Stock solution of Streptomycin sulfate salt (Sigma-Aldrich®) was filter-sterilized by passing through a nitrocellulose membrane filter (0.22 μm) before using in the experimental systems.

2.2. Experimental protocol

The experimental culture units were glass bottles that were inoculated *Chlorella vulgaris* culture in the exponential phase. Streptomycin (Sigma-Aldrich®) was added from a stock solution to reach the desired concentration in the culture medium. The concentrations used were: 0, 0.3, 0.6, 1.2, 1.8 and 2.4 mg L^{-1}. The effects on growth and metabolism of *Chlorella vulgaris* were measured 72 h following exposure to the antibiotic. Experiments were performed with 3–6 replicates, n = 3–6.

2.3. Growth and metabolic measurements

(a) Growth:
Microalgae growth was quantified as dry weight. A known volume of the alga culture from each treatment was filtered on a 5 μm pore nitrocellulose membrane and dried at 80 °C for 72 h to constant weight. The percentage of growth inhibition in relation to the control was derived as the difference between control and treatment.

(b) Oxygen evolution:
Photosynthetic oxygen evolution rates were measured with a temperature-controlled Clark-type oxygen electrode (Oxygraph, Hansatech, Instruments Ltd., Norfolk, UK) in exponentially grown microalgae cultures. A known volume of the alga culture was concentrated by centrifugation (3500 rpm for 1 min) and resuspended in fresh medium. The metabolic activity was measured in the same medium by turning on and off a white actinic light (400 μmoles m^{-2} s^{-1}). Chlorophyll *a* and *b* and total carotenoids were determined spectroscopically with 100% (v/v) methanol extracts (Wellburn, 1994).

(c) Photosynthetic activity: Chlorophyll a fluorescence transients
Chlorophyll *a* fluorescence transients were measured at laboratory temperature with a Handy-Plant Efficiency Analyzer (HPEA, Hansatech, Instruments Ltd., Norfolk, UK) as described in Strasser (1997). Each chlorophyll *a* fluorescence induction curve was analyzed according to the JIP-test using the BiolyzerHP3 software (Strasser and Strasser, 1995). The following data from the original measurements were used: (1) the minimal fluorescence yield Fo at 50 μs, (2) the maximal fluorescence yield, Fm, (3) the initial slope at the beginning of the variable fluorescence, $Mo = 4 \cdot (F_{300\ \mu s} - Fo)/(F_M - Fo)$, (4) the relative fluorescence at 2 ms (phase J), (5) the variable fluorescence at phase J, $V_J = (F_J - F_O)/(F_M - F_O)$ and (6) the variable fluorescence at 300 μs; $V_K = (F_{300\ \mu s} - F_{50\ \mu s})/(F_M - F_{50\ \mu s})$. Using these data, the following fluxes ratios and parameters were calculated following Appenroth et al. (2001):

1. The maximum quantum yield of PSII for primary photochemistry; $\phi Po = Fv/Fm$
2. The efficiency with which a trapped exciton, having triggered the reduction of Q_A to Q_A^- can move an electron further than Q_A^- into the electron transport chain; $\psi_O = 1 - V_J$;
3. The quantum yield of electron transport; $\phi Eo = Fv/Fm \cdot \psi_O$
4. The amount of active PSII reaction centers per absorption; $RC/ABS = [(1 - (F_O - /F_M))](M_O/V_J)$
5. Performance index on absorption basis: $PI_{(ABS)} = (RC/ABS) \times (\phi Po/(1 - \phi Po)) \times (\psi_O/(1 - \psi_O))$

The index is formed of three separate terms: (1) the concentration of active reaction center chlorophyll [ABS/RC/(1 − ABS/RC)], (2) an expression related to primary photochemical [ϕPo/(1 − ϕPo)] and (3) an expression related with the electron transport after Q_A [Ψo/(1 − Ψo)].

(d) Photosynthetic activity: Relative electron transport and Non photochemical quenching.
The relative electron transport rate (rETR) and the non-photochemical quenching (NPQ) were measured using PAM fluorometer (FMS 2 Hansatech Instruments Ltd., Norfolk, UK). All measurements were performed at laboratory temperature in the dark. The algal samples were adjusted at same optical density ($0.4_{750\ nm}$) before the chlorophyll fluorescence study. Each cell suspension was filtrated through a nitrocellulose filter (5 μm) and then they were dark adapted for 15 min (Mallick and Mohn, 2003). The relative electron transport rate (rETR) was calculated using the formula $rETR = [(\Phi PSII) \cdot (200\ \mu moles\ m^{-2}\ s^{-1})]$, and the non photochemical quenching (NPQ) according to Sperdouli and Moustakas (2012). $\Phi NPQ = 1 - \Phi PSII - [1/NPQ + 1 + ((qL^*(Fm/Fo - 1)))]$; where: $\Phi PSII = (Fm' - Fs)/Fm'$; $qL = [(Fm' - Fs)/(Fm' - Fo')]^*(Fo'/Fs)$; $NPQ = (Fm - Fm')/Fm'$;.