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Effect of chromium on the fatty acid composition of two strains of *Euglena gracilis*

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Fatty acid evaluation in the presence of chromium in Euglena gracilis grown in different culture conditions.

Abstract

The effect of hexavalent chromium on fatty acid composition was studied in two strains of *Euglena gracilis*; UTEX 753 (from the Culture Collection of Algae of Texas University, USA) and MAT (isolated from a highly polluted River). Both were grown in photoauxotrophic and photoheterotrophic conditions and exposed to two metal concentrations, one below and one above IC_{50}. The high malondialdehyde (MDA) levels (3 to 7-fold) obtained with chromium concentration above IC_{50}, suggested the existence of metal-induced lipid peroxidation. Total lipid content increased only with concentration below IC_{50}, whereas it was inhibited by higher metal concentration. Photoheterotrophic control strains exhibited a significantly higher proportion of saturated and polyunsaturated fatty acids. Polyunsaturated acids were most affected by chromium, especially those related to chloroplast structures. Ultra-structure studies showed clear thylakoid disorganization in all treated cells. The results indicate that hexavalent chromium affects levels of fatty acids, especially those related to photosynthetic activity.

Keywords: *Euglena gracilis*; Fatty acids; Hexavalent chromium; Heavy metal; Lipids

1. Introduction

Hexavalent chromium is a highly toxic heavy metal that seriously affects environmental conditions in many aquatic ecosystems (Bagchi et al., 2002). The toxicity of this metal depends on its physicochemical properties. The oxidation properties of ion CrO_4^{-2} and its structural similarity to inorganic anions allow chromium to easily go through cell membranes, thus being an alternative substrate in the sulfate transport system (Cieslak-Golonka, 1996; Haglund, 1997). The cytotoxic effects of this metal on animals and plants are well documented, and its mutagenicity turn it into the source of different types of human cancer (Vajpayee et al., 2001; Bagchi et al., 2002). Chromium discharges into surface water carried out by electroplating units, as well as textile, leather tanning, and paper industries increased its concentration several times above natural levels.

It is well known that algal cells exposed to heavy metals may suffer serious morphological and biochemical alterations (Devars et al., 1998; Rai and Rai, 1998; Okamoto et al., 2001). The reported effects of hexavalent chromium on algal cells include pigment content reduction, chloroplast disorganization, mitochondrial damage, and cytoskeleton alterations causing loss of mobility and cellular growth another mechanism of inhibition is affecting DNA (Wang, 1999; Cervantes et al., 2001; Rocchetta et al., 2003).

Changes in the lipid composition of algae and plants have been attributed to the variations in environmental or culture conditions, (Molina Grima et al., 1994; Harwood, 1995; Ramalho et al., 1998; Chini Zittelli et al., 1999). Studies on the

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effect of different growth conditions performed on fatty acids from *Euglena gracilis* strains showed that exogenous carbon has an enormous influence on lipid class and fatty acid composition (Regnault et al., 1995; Barsanti et al., 2000).

It has been demonstrated that polyunsaturated fatty acids are the main targets of free radicals in lipid peroxidation (Girotti, 2001). Einicker-Lamas et al. (1996) reported several changes in membrane lipid content when *Euglena gracilis* cells were cultured in the presence of cadmium.

The unicellular protist *Euglena gracilis* is a useful model to study cell damage caused by cytotoxic compounds such as heavy metals (Coppellotti, 1989; Navarro et al., 1997). This microorganism grows in photoauxotrophic conditions like most green euglenoids, but it is also able to develop in heterotrophic or photoheterotrophic conditions.

The aim of our study was to examine fatty acid composition and content in two strains of *E. gracilis*; MAT (isolated from the highly polluted Matanza River, Buenos Aires, Argentina) and UTEX 753 (from the Culture Collection of Algae of Texas University, USA). Strains were cultured under photoauxotrophic and photoheterotrophic conditions and exposed to different chromium concentrations. Effects on morphology and total lipid content were analyzed, and lipid peroxidation was evaluated in terms of MDA content.

2. Materials and methods

2.1. Microorganism and culture conditions

The strains of *E. gracilis* used were: UTEX 753, from the Culture Collection of Algae of Texas University, USA (generously provided by Dr Richard Triemer), and MAT, isolated from the Matanza River, Buenos Aires, Argentina (Ruiz et al., 2004). Experimental cultures were grown in two mineral media; Cramer & Myers (C&M) and Buetow (with sodium acetate as carbon source) (Buetow, 1982), at 24 ± 1 °C, with cool-white fluorescent continuous light (150 μE m^{-2} s^{-1} irradiance). Axenicity was monitored plating cultures in a bacterial medium. A new culture was initiated 6 days before each experiment in order to obtain an inoculum in exponential growth.

2.2. Metal toxicity assays

Experiments were performed on static cultures containing 150 ml culture medium in 250 ml glass flasks, at 24 ± 1 °C, and under cool-white fluorescent continuous light, with an irradiance of 150 μE m^{-2} s^{-1}. Aliquots of 10^5 cells ml^{-1} from both stock cultures (MAT and UTEX) were inoculated in each flask. $K_2Cr_2O_7$ was added axenically from a 0.1 M stock solution.

The IC_{50} values obtained in a previous study for MAT and UTEX strains cultured in Buetow medium were 24.6 μM Cr(VI) and 3.2 μM Cr(VI), respectively. On the other hand, IC_{50} values observed in the strains grown in C&M medium were 120.5 μM Cr(VI) for MAT and 90.4 μM Cr(VI) for UTEX. Growth rates for the conditions assayed were quite different not only between strains but also between culture media. Since IC_{50} values were also different, we decided to express concentrations as a percentage (40% and 80%) respect to the concentration necessary to obtain 50% growth (IC_{50}). For MAT strain cultured in Buetow medium, the concentrations corresponding to 40% and 80% were 9.8 μM and 19.7 μM, respectively, while for cells grown in C&M medium, they were 48.2 μM for 40% and 96.4 μM for 80%. In the case of UTEX, values were 1.3 μM and 2.6 μM for cells cultured in Buetow medium, and 36.2 μM and 72.3 μM for samples cultured in C&M medium.

Cellular density was determined with a Neubauer chamber, with less than 10% error, $P < 0.05$. Harvesting time was 96 h after start of metal stress for all the cultures (U.S. Environmental Protection Agency, 1985).

2.3. Transmission electron microscopy

Cells collected by centrifugation at 4500 × *g* for 20 min were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Then, they were post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 2 h, dehydrated in an acetone series, and embedded in Spurr resin. Ultrathin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Later, they were examined using a JEOL 100 CX-II electron microscope from the Basic and Applied Investigation Center of Bahía Blanca, Argentina.

2.4. Total lipid determination

Cultures cells were harvested by centrifugation for 15 min at 4500 × *g* and washed three times with 0.154 M phosphate buffer, pH 7. Total lipids were extracted with chloroform:methanol (2:1 v/v) and quantified according to the Bligh and Dyer method (1959).

2.5. Malondialdehyde (MDA) determination

Cultures were harvested by centrifugation for 15 min at 4500 × *g*, and washed three times with 0.154 M phosphate buffer, pH 7. Lipid peroxidation levels were measured in terms of malondialdehyde (MDA) content determined by the thiobarbituric acid reactive substances (TBARS) method (Vavilin et al., 1998), according to Hodges et al. equations (1999).

2.6. Fatty acid determination

Dried biomass (15 mg) was exposed to direct transesterification with 1 ml acetyl chloride in methanol 1:20 (v/v), according to Lepage and Roy (1984). After tubes were cooled in water (25 °C), the reaction mixture was diluted with 1 ml water, and extracted three times with 1 ml hexane. The hexane phase was dried under gentle nitrogen stream, at atmospheric pressure and room temperature. Then, fatty acid methyl esters were resuspended in 100 μl hexane and injected into the chromatograph. Composition assessment was performed using a gas chromatograph (Hewlett Packard GC5890) with a flame ionization detector, on an Innowax capillary column (30 m, 0.32 mm ID, 1 μm film thickness). Helium was used as carrier gas. The column was held at 150 °C for 3 min, and then temperature was increased 5 °C/min until reaching 280 °C (held for 15 min). Injection port and detector temperatures were 250 °C and 280 °C, respectively. Determinations were carried out in triplicate. A standard of fatty acid methyl ester mixture (Supelco Inc., Supelco Park, Bellfonte) was run under identical conditions to identify compounds on the basis of their retention times.

Fatty acid quantitation was performed using heptadecanoic acid (C17:0) as internal standard. Thus, an aliquot of 125 μg heptadecanoic acid dissolved in 5 μl toluene was added to the biological samples before transesterification.

2.7. Statistical analysis

Quantitative data for fatty acids content represent the mean of three independent experiments. The statistical significance of differences between controls and treated cultures and between Buetow and C&M media was determined by an analysis of variance (ANOVA). For MDA and total lipid contents, mean and standard deviation were obtained from the duplicates of each concentration. Each treatment was performed in duplicate and each assay was repeated three times. Data were evaluated by an analysis of variance (ANOVA). Values of $P < 0.005$ were considered significant.

3. Results

Fig. 1 shows ultrastructural morphology differences between control cells (Fig. 1A) and cells grown in the presence of the higher chromium concentration (Fig. 1B). Independently of the culture media used, the main damages observed in both

strains were significant chloroplast thylakoid disorganization and the presence of several vacuoles. Mitochondria, on the other hand, did not seem to be affected by chromium.

Fig. 2 describes the important variation of total lipid content detected between controls grown in Buetow and C&M media. Both control and treated cells cultured in Buetow (a mineral medium with sodium acetate as carbon source) presented higher lipid levels than those cultured in C&M. They were greater in MAT strain. On the other hand, the lower chromium concentration increased total lipid content respect to control cells, whereas the higher decreased it.

Fig. 3 shows malondialdehyde (MDA) content in cells treated with chromium. Independently of the culture medium used, all treated cells exhibited increased MDA levels according to the metal concentration assayed. The highest levels were observed in control and treated cells grown in C&M medium.

Table 1 describes variations in fatty acid content according to the different conditions assayed. UTEX controls cultured in Buetow medium showed a significantly higher proportion of saturated fatty acids (SAFAs) and polyunsaturated fatty acids (PUFAs) than cells grown in Cramer & Myers medium. Among saturated fatty acids, myristic (14:0) and palmitic (16:0), and among unsaturated fatty acids, linoleic (18:2ω6) and arachidonic (20:4ω6) were the ones that most contributed to the differences mentioned above. UTEX control cells grown in Buetow medium exhibited significantly ($P < 0.005$) higher ω6 content and lower ω3 content than cells cultured in C&M medium. On the other hand, MAT control cells cultured in Buetow medium showed significantly ($P < 0.005$) higher myristic acid content (14:0) than those grown in C&M medium. This accounted for the difference in SAFA content observed between cells grown in Buetow (19.5%) and in C&M medium (13.6%).

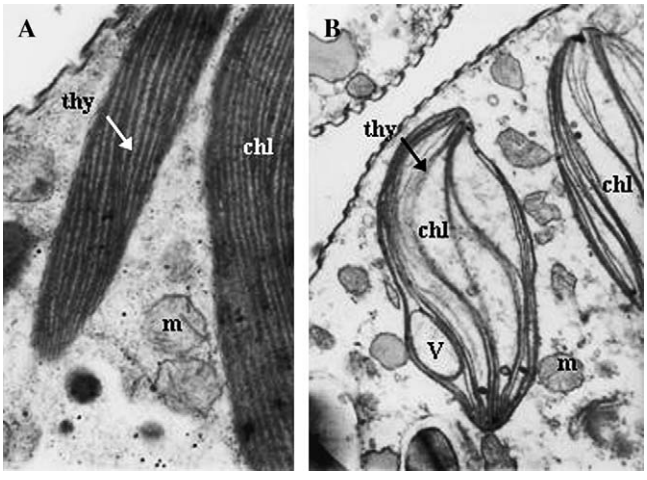

Fig. 1. Transmission electron microscopy of *Euglena gracilis* MAT cultured in Cramer & Myers medium. A: Control cells. B: Cells treated with the higher chromium concentration. Both strains, grown in the two culture media, showed similar chloroplast damage in the presence of the higher metal concentration. Both strains, independently of the culture medium used, showed significant thylakoid alteration (see arrow) and high number of vacuoles. Mitochondria (m), chloroplast (chl), thylakoids (thy) (arrow), vacuoles (V). Bars = 0.5 μm (A) Bars = 0.1 mm (B).

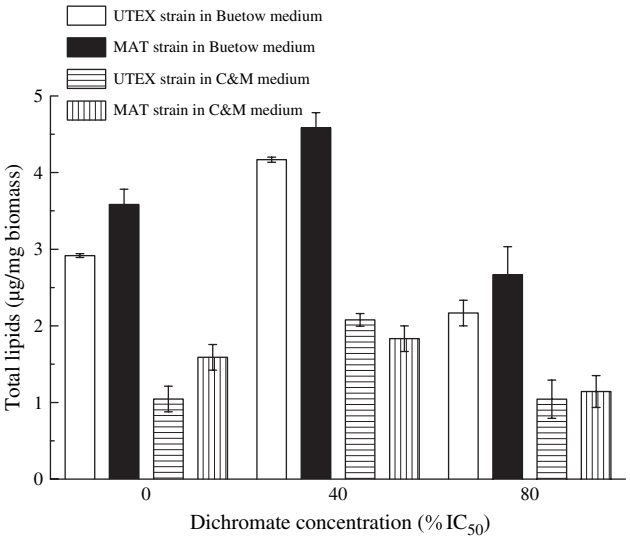

Fig. 2. Total lipid content in two strains of *Euglena gracilis* cultured in Buetow and Cramer & Myers media in the presence of two chromium concentrations. Concentrations are expressed as percentage (40% and 80%) of the concentration necessary to obtain 50% growth (IC_{50}). Results are expressed as mean ± SD of three different experiments.

Both UTEX and MAT control cells exhibited differences in fatty acid content when they were cultured in Buetow medium. MAT cells showed higher saturated fatty acid (SAFA) levels and lower polyunsaturated fatty acid (PUFA) levels, due to the important differences in 14:0, 18:0 and 20:4ω6 fatty acid content. No significant differences were observed between control cells from both strains grown in C&M medium.

The two strains were affected by chromium exposure, being PUFAs (especially ω3 and ω6) the most damaged fatty acids. Among them, linolenic acid (18:3ω3) suffered a significant ($P < 0.005$) decrease when strains were exposed to the

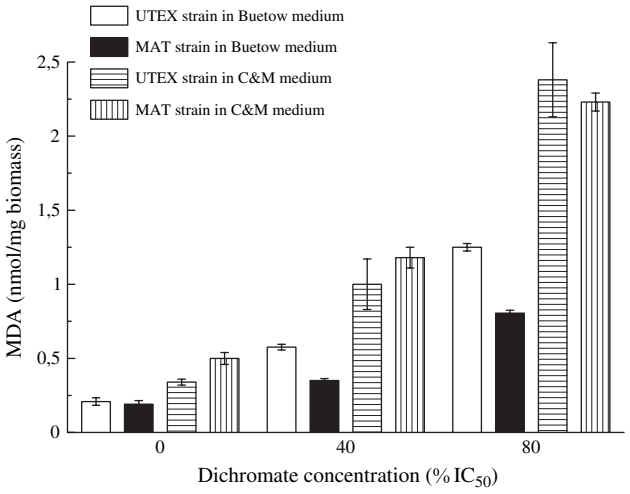

Fig. 3. Malondialdehyde (MDA) content in two strains of *Euglena gracilis* cultured in Buetow and Cramer & Myers media in presence of two chromium concentrations. Concentrations are expressed as percentage (40% and 80%) of the concentration necessary to obtain 50% growth (IC_{50}). Results are expressed as mean ± SD of three different experiments.

Table 1
Fatty acid composition (% total fatty acids) of *Euglena gracilis* MAT and UTEX grown in Buetow and Cramer and Myers culture media, in presence of two different chromium concentrations. They are expressed as percentage (40% and 80%) respect to the minimal concentration necessary to obtain 50% growth (IC_{50}). (n.d. = not detectable)

	UTEX strain grown in Buetow medium			UTEX strain grown in Cramer and Myers medium		
	Control	40% IC_{50}	80% IC_{50}	Control	40% IC_{50}	80% IC_{50}
C14:0	4.5 ± 0.4	7.9 ± 1.9	9.4 ± 1.7[a]	1.7 ± 0.1[b]	2.9 ± 0.2[b]	1.6 ± 0.1[b]
C16:0	10.1 ± 1.1	9.4 ± 0.7	10.5 ± 0.7	6.3 ± 0.4[b]	8.2 ± 1.0	6.6 ± 0.6[b]
C16:1ω7	1.0 ± 0.3	1.1 ± 0.1	1.4 ± 0.2	0.8 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
C16:2ω4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C16:3ω4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:0	1.8 ± 0.1	2.7 ± 0.6	2.4 ± 0.8	3.0 ± 0.6	3.6 ± 0.6	3.4 ± 0.7
C18:1ω9	6.1 ± 0.6	6.5 ± 0.1	5.9 ± 0.5	4.6 ± 0.1	6.3 ± 0.1	5.8 ± 0.9
C18:1ω7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:2ω6	5.8 ± 0.6	5.5 ± 1.1	3.8 ± 0.7[a]	3.8 ± 0.1[b]	4.1 ± 0.5	3.5 ± 0.3
C18:3ω4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:3ω3	10.9 ± 1.5	8.6 ± 1.7	7.6 ± 1.5[a]	10.4 ± 2.0	6.9 ± 0.8[a]	6.1 ± 0.8[a]
C18:4ω3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:1ω9	n.d.	n.d.	n.d.	n.d.	n.d.	0.5 ± 0.1
C20:4ω6	5.3 ± 0.9	4.0 ± 1.0	3.6 ± 0.8	1.4 ± 0.1[b]	1.9 ± 0.3[b]	1.9 ± 0.1[b]
C20:4ω3	1.2 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.5 ± 0.2	1.6 ± 0.1	1.9 ± 0.8
C20:5ω3	2.9 ± 1.0	2.9 ± 0.7	3.2 ± 0.7	3.0 ± 0.2	2.9 ± 0.6	3.4 ± 0.5
C22:5ω3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:6ω3	n.d.	n.d.	n.d.	1.0 ± 0.1	n.d.	n.d.
ω3	14.9	12.8	12.1[a]	16.2[b]	11.4[a]	11.4[a]
ω6	11.1	9.5	7.4[a]	5.2[b]	6.0[b]	5.3[b]
Σ SAFA	15.0	20.0[a]	22.2[a]	11.0[b]	14.7[b]	11.5[b]
Σ MUFA	7.0	7.6	7.5	5.5	7.4	7.2
Σ PUFA	26.0	22.3	19.5[a]	21.7[b]	17.4[b]	16.8[a]
Σ SAFA/(Σ MUFA + Σ PUFA)	0.5	0.7[a]	0.8[a]	0.4	0.6	0.5

	MAT strain grown in Buetow medium			MAT strain grown in Cramer and Myers medium		
	Control	40% IC_{50}	80% IC_{50}	Control	40% IC_{50}	80% IC_{50}
C14:0	8.5 ± 2.8[c]	9.1 ± 0.5	8.9 ± 0.4	2.4 ± 0.6[b]	1.9 ± 0.3[b]	2.1 ± 0.2[b]
C16:0	8.6 ± 1.7	11.1 ± 0.6	11.0 ± 0.2	8.4 ± 1.2	9.8 ± 1.7	9.2 ± 0.5[b]
C16:1ω7	1.2 ± 0.3	1.0 ± 0.1	1.0 ± 0.3	1.3 ± 0.1	1.5 ± 0.4	1.1 ± 0.1
C16:2ω4	n.d.	n.d.	0.2 ± 0.1	0.4 ± 0.1	n.d.	n.d.
C16:3ω4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:0	2.4 ± 0.1[c]	3.2 ± 0.2	3.9 ± 0.3[a]	2.8 ± 0.7	3.4 ± 0.5	4.5 ± 0.4[a]
C18:1ω9	4.0 ± 0.2	4.0 ± 0.1	4.7 ± 0.5	3.9 ± 0.5	3.9 ± 0.3	4.7 ± 0.1
C18:1ω7	n.d.	1.2 ± 0.2	n.d.	n.d.	n.d.	n.d.
C18:2ω6	5.4 ± 0.8	4.3 ± 0.2	2.4 ± 0.2[a]	4.7 ± 0.2	3.5 ± 0.4	1.9 ± 0.3[a]
C18:3ω4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:3ω3	7.7 ± 1.7	7.6 ± 0.3	5.8 ± 0.7[a]	10.5 ± 1.1	8.1 ± 0.4	6.0 ± 0.6[a]
C18:4ω3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:1ω9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:4ω6	2.8 ± 0.5[c]	2.9 ± 0.2	2.7 ± 0.4	2.3 ± 0.1	2.1 ± 0.5	1.8 ± 0.1[b]
C20:4ω3	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.3
C20:5ω3	3.5 ± 1.0	2.8 ± 0.3	2.7 ± 0.5	3.4 ± 0.5	3.2 ± 0.5	2.2 ± 0.6
C22:5ω3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:6ω3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ω3	11.8	11.0	9.2[a]	14.6[b]	11.9	8.8[a]
ω6	8.1	7.2	5.1[a]	7.0[b]	5.6[b]	3.6[a,b]
Σ SAFA	19.5[c]	23.4[a]	23.9[a]	13.6[b]	15.1[b]	15.7[b]
Σ MUFA	5.2	6.2	5.7	5.1	5.4	5.7
Σ PUFA	20.2[c]	18.4	14.4[a]	21.9	17.5	12.4[a]
Σ SAFA/(Σ MUFA + Σ PUFA)	0.8	1.0[a]	1.19[a]	0.5	0.7	0.9[a]

Data are mean ± SD of three independent experiments.
[a] Denotes significant ($P < 0.05$) analysis of variance (ANOVA) between control and treated cells.
[b] Denotes significant ($P < 0.05$) analysis of variance (ANOVA) between both culture media, Cramer and Myers medium respect to Buetow medium.
[c] Denotes significant ($P < 0.05$) analysis of variance (ANOVA) between control cells of both strains.

highest chromium concentration, independently of the medium assayed. Regarding ω6 content, linoleic acid (18:2ω6) was the fatty acid most affected in almost all the conditions assayed. In contrast, SAFAs (14:0 for UTEX strain and 18:0 for MAT strain) showed an important increase related to chromium exposure only in the strains cultured in Buetow medium. In spite of the significant ($P < 0.005$) increase observed in 18:0 content for MAT strain grown in C&M medium, SAFA content showed no important differences.

Ratio variation between saturated and unsaturated fatty acids according to chromium levels is shown in Table 1. The ratio increased with metal concentration due to high SAFA content and low unsaturated fatty acid content, being more remarkable in MAT strain, independently of the culture medium used.

4. Discussion

Lipids related to chloroplast structures such as linoleic (18:2ω6) and linolenic acids (18:3ω3) (Barsanti et al., 2000) were the most affected by chromium, especially those from strains grown in C&M medium. This agrees with data found on MDA levels, which were higher in treated cells cultured in this medium. A previous study reported a decrease in chlorophyll levels at different chromium concentrations (Rocchetta et al., 2003) that could be related with chloroplast disorganization observed in Fig. 1B. Some authors described important mitochondria and chloroplast alterations caused by several heavy metals in *Euglena gracilis* (Einicker-Lamas et al., 2002; Mendoza-Cozatl et al., 2002). However, we detected only a few mitochondrial alterations. Moreover, fatty acids related with non-photosynthetic structures, such as the mitochondrial system and the microsomes (C20:4ω6 and C20:5ω3) (Barsanti et al., 2000) showed no significant differences after chromium treatment. This suggests that chloroplasts would be the target organelle of chromium toxicity in *E. gracilis* strains, in accordance with reports from different authors (Duret et al., 1986; Einicker-Lamas et al., 1996).

Our results indicate that differences in fatty acid content depend on culture media. Carbon source plays an important role in the synthesis of *Euglena gracilis* lipids (Regnault et al., 1995). Control cells of both strains grown in a mineral medium with exogenous carbon source (like acetate in Buetow medium) showed higher SAFA content rich in myristic (C14:0) and palmitic (C16:0) acids, which are storage lipids (Regnault et al., 1995). Among PUFAs related to chloroplasts, the most abundant fatty acid measured was linolenic acid (18:3ω3) for all the condition assayed.

Cells grown in C&M medium (without exogenous carbon source) showed higher ω3 and lower ω6 than cells cultured in Buetow medium. This may be due to the metabolic versatility shown by *E. gracilis*, which can follow plant or animal synthetic pathways (Buetow, 1989). Growth conditions are very important since cells cultured in the medium without an exogenous carbon source are more dependent on photosynthetic activity and, therefore, produce higher levels of fatty acids rich in ω3 (Barsanti et al., 2000). This may explain why the levels of malondialdehyde, MDA, (a lipid oxidation product) were greater in controls from both strains grown in C&M than in Buetow. This difference may be due to a higher photosynthetic activity, which would lead to an increase of reactive oxygen species (ROS), generating a more oxidative intracellular environment (Watanabe et al., 2003).

Both strains cultured in Buetow medium presented differences in fatty acid content (Table 1). MAT showed lower PUFA content and a higher SAFA content (C14:0, C18:0) than UTEX, indicating higher storage lipid content. This agrees with total lipid levels, which were higher in MAT control cells. MAT (isolated from a highly polluted River) could have reduced biomembrane PUFA content and increased SAFA levels in order to adapt itself to an oxidative environment, protecting its intracellular membrane structure and cellular functions (Watanabe and Suzuki, 2002).

Fatty acid content was clearly affected by chromium exposure. Saturated fatty acids showed an important increase in treated cells grown in Buetow medium. This might be due to the ability of cells to incorporate carbon from the medium to form storage lipids rich in C14:0, C16:0 and C18:0 (Regnault et al., 1995). These saturated acids could be used later in PUFA synthesis to counteract their decrease caused by oxidative stress (Harwood, 1988).

In spite of the significant decrease observed in PUFA content in all the cells treated with the higher metal concentration (specially ω3 and ω6 type, Table 1), MDA levels showed lipid oxidation even at the lower concentration (Fig. 3). Moreover, total lipids showed a significant increase with this concentration, may be as a defense mechanism to counteract oxidative damage. Einicker-Lamas et al. (1996) reported an increase in total lipids due to a higher concentration of cholesterol and phospholipid, or other lipid compounds, possibly related to a detoxification mechanism. Lipid levels decreased with the higher concentration showing irreversible damage.

Ratio variation between saturated and unsaturated fatty acids (Table 1) was based on the increase of SAFAs suffered by cells cultured in Buetow medium and the decrease of PUFAs at all the conditions assayed, showing marked differences in cellular metabolism depending on culture conditions.

These results indicate that hexavalent chromium can produce lipid peroxidation and affect the content of fatty acids. Those related with the photosynthetic activity are the most damaged ones. The oxidative modification of fatty acid content could play an important role. Changes in SAFA and PUFA levels caused by metal treatment could be part of a defense or reparation mechanism aimed at reducing cellular damage caused by hexavalent chromium stress.

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