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Effect of different nutrient concentrations on growth and hepatotoxin production by *Microcystis aeruginosa* (Cyanobacteria)

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Introduction

Increasing eutrophication of Brazilian fresh and brackish water-bodies, mainly close to urban centres, has allowed Cyanobacteria to become dominant in these environments. This problem is intensified due to the fact that most Brazilian water supplies have the environmental characteristics necessary for cyanobacterial growth during the entire year. The most common cyanobacterial genus producing blooms on the surface of eutrophic Brazilian water-bodies is *Microcystis* (Tundisi & Matsumara-Tundisi 1992) and the hepatotoxin production by several Brazilian cyanobacterial strains has been confirmed by Costa & Azevedo (1994). In order to understand the relationship between nutrient concentration and hepatotoxin production, a hepatotoxic strain (NPJB-1) of *Microcystis aeruginosa* isolated from Lagoa das Garças (São Paulo city, Brazil), was cultivated in different nutrient concentrations to analyse the variation and the relationship between cellular growth and toxin production.

Methods

The strain of NPJB-1 was maintained in culture conditions as follows: 30 µmol photon. m^{-2} s^{-1} of light; photoperiod of 12 h; 21 ±2 °C of temperature; ASM-1 medium, using different concentrations of the nutrients: ½ASM-1 (medium with nutrient concentrations reduced by half); ASM-1 (control condition); 2ASM-1 (medium with nutrient concentrations duplicated); 5ASM-1 (medium with nutrient concentrations increased 5 times). Culture growth was monitored by cell count, growth rates, biomass production and cell yield. Cells growing in the exponential phase were harvested, concentrated by centrifugation, and lyophilized for mouse bioassays to determine the lethal dose for 50 % of the animals (LD50), and toxin extraction. All experiments were performed with three replicates of each growth condition, here presented as average values. The bioassays were done using intraperitoneal injections (i. p.) on Swiss male mice (15–25 g). Freeze dried cells were suspended in 0.99 % NaCl solution and three mice were injected per dose level. Signs of poisoning, time of death and liver weight were noted. Extraction, isolation and purification of the toxins were carried out by reversed phase HPLC and HPLC-PicoTag amino acid analysis, using a slightly modified method of Krishnamurthy et al. (1986). Characterization was done by fast atom bombardment mass spectrometry (FAB-MS) in the same conditions described by Azevedo et al. (1994).

Results

Growth rates per day (K), biomass (µg/10^6 cell) and cell yield (10^6 cell/mL) were respectively: 0.08–3.0–12.3 (1/2ASM-1); 0.12–3.7–19.2 (ASM-1); 0.18–6.4–26.4 (2ASM-1) and 0.14–8.3–23.3 (5ASM-1). The LD50s were respectively: 85, 30, 168 and 345 mg kg^{-1}. As described by Azevedo et al. (1994), this *Microcystis aeruginosa* strain (NPJB-1) produces two different hepatotoxic heptapeptides: Microcystin – LR and LF. The stimulation on cell growth and biomass production, by increase of nutrients in the culture medium, did not show a straight relationship with toxin production. The decrease of hepatotoxicity under conditions ½ASM-1 and 2ASM-1 was mainly related to decreasing Microcystin – LR production, analysed by HPLC chromatograms.

Discussion

These data suggest that an increase in nutrient concentrations in aquatic environments would not be directly related to hepatotoxin production by *Microcystis aeruginosa*. However, the number of toxic cells can be increased under eutrophic conditions by higher cellular growth rates. The potential for human poisoning increases since reports by different authors have confirmed that microcystin – LR produced by several strains of this species is a potent tumour promoter that inhibits protein phosphatase type 1 (PP1) and type 2A (PPA). Furthermore, bioaccumulation of the microcystins by some

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aquatic invertebrates has been confirmed. These findings show that it is necessary to monitor the occurrence of toxic cyanobacteria and their toxins in Brazilian natural freshwater supplies and reservoirs, as well as verify the possible bioaccumulation of these toxins in the food chain.

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