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Occurrence of saxitoxins and an anatoxin-a(s)-like anticholinesterase in a Brazilian drinking water supply

Renato J.R. Molica[a,*], Eduardo J.A. Oliveira[b], Paulo V.V.C. Carvalho[c], Anapaula N.S.F. Costa[c], Maristela C.C. Cunha[a], Gustavo L. Melo[a], Sandra M.F.O. Azevedo[d]

[a]*Instituto de Tecnologia de Pernambuco (ITEP), Laboratório de Ecofisiologia de Microalgas, Av. Prof. Luis Freire 700, Recife-PE 50.740-540, Brazil*
[b]*Centro Federal de Educação Tecnológica de Pernambuco, (CEFET), Av. Prof. Luis Freire 500, Recife-PE 50740-540, Brazil*
[c]*Companhia Pernambucana de Saneamento – COMPESA, Gerência de Controle de Qualidade, Largo Dois Irmãos 1012, Recife-PE 52.071-440, Brazil*
[d]*Instituto de Biofísica Carlos Chagas Filho, CCS, Bloco G, UFRJ, Ilha do Fundão, Rio de Janeiro 21949-900, Brazil*

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Abstract

Blooms of toxic cyanobacteria are very common in Brazilian waterbodies, as a consequence of eutrophication processes. Our investigations were focused on the detection of neurotoxins during a cyanobacterial bloom in Tapacurá reservoir, which serves as a water supply for Recife city in northeastern Brazil. We also investigated the possible presence of neurotoxins in strains of *Anabaena spiroides* isolated from this environment. Samples were collected from March to May 2002 at the water surface and close to the dam. Limnological parameters (conductivity, pH, inorganic nutrients) and cyanobacterial abundance were measured. The samples were assayed for toxicity by mouse bioassay and acetylcholinesterase-inhibiting activity by a colorimetric method; saxitoxins (paralytic shellfish poisons) were quantified by a HPLC-FLD postcolumn derivatization method. The dominant cyanobacteria during the bloom were found to be *A. spiroides*, *Pseudanabaena* sp., *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa*. The mouse bioassays showed the presence of neurotoxins during both *A. spiroides* and *C. raciborskii* dominance, whereas anticholinesterase activity was only observed during periods of *A. spiroides* dominance. The *A. spiroides* strains isolated during the study also exhibited an acetylcholinesterase inhibitor. HPLC-FLD chromatograms of bloom material extracts revealed the presence of saxitoxin, neosaxitoxin and dc-saxitoxin, probably produced by *C. raciborskii*.

Keywords: Acetylcholinesterase inhibitor; *Anabaena spiroides*; Anatoxin-a(s); Cyanobacterial bloom; *Cylindrospermopsis raciborskii*; Saxitoxins

* Corresponding author. Tel.: +55 81 3272 4264; fax: +55 81 3272 4287.
E-mail address: renato@itep.br (Renato J.R. Molica).

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1. Introduction

Cyanobacterial blooms are very frequent events in Brazilian drinking water supplies, since most of them are eutrophic or hypereutrophic (Souza et al., 1998; Huszar et al., 2000; Sant'Anna and Azevedo, 2000). This situation is most pronounced in the northeast of Brazil, a region subjected to recurrent periods of drought and therefore, with a large number of surface reservoirs to store water (Bouvy et al., 1999).

Cyanobacterial toxins (cyanotoxins) can be grouped either as hepatotoxins or neurotoxins, according to the targets of their toxic actions. The first includes microcystins and nodularins, cyclic peptides that inhibit some protein phosphatases and cylindrospermopsin, an alkaloid suppressor of protein synthesis. Neurotoxins include anatoxin-a, a post-synaptic cholinergic nicotinic agonist, anatoxin-a(s), an inhibitor of acetylcholinesterase activity and saxitoxins (paralytic shellfish poisons), sodium channel blockers in the nerves (Chorus and Bartram, 1999).

The cyanotoxins already known to occur in Brazil are microcystins (Azevedo et al., 1994; Matthiensen et al., 2000), saxitoxins (Lagos et al., 1999) and anatoxin-a(s)-like toxin(s) (Monserat et al., 2001). Cylindrospermopsin was detected only once in a carbon filter from a hemodialysis clinic in Caruaru (Carmichael et al., 2001) and anatoxin-a has never been reported. The majority of these studies have investigated strains and field samples taken from estuaries or ornamental lakes and very few have investigated the presence of cyanotoxins during cyanobacterial blooms in drinking water supplies.

The development of these organisms in waterbodies of Pernambuco State, northeastern Brazil, is widespread (Bouvy et al., 2000). However, the knowledge about which toxins occur, as well as which species could be toxin producers, is still scarce.

In this report, we present the occurrence of saxitoxins and anatoxin-a(s)-like toxin(s) during a cyanobacterial bloom in a Brazilian drinking water supply. Tapacurá is a eutrophic reservoir located in Pernambuco State (8°02′S and 35°09′W). At full capacity its volume is 94.2×10^6 m^3 and it is used as water supply for approximately 1.35 million inhabitants (Braga, 2001; Bouvy et al., 2003). We also have investigated the occurrence of neurotoxins in isolates of *Anabaena spiroides* Klebahn from this reservoir.

2. Materials and methods

2.1. Sampling site, analytical procedures and nutrients

The Governmental water company has been developing a water-monitoring program in Tapacurá reservoir since 2001 including cell counts of cyanobacteria, toxicity measurements and some limnological parameters.

Sampling was carried out weekly from 19 March to 10 April 2002 and from 8 May to 30 May 2002 at the surface close to the dam of Tapacurá reservoir. The conductivity and pH were recorded using specific electrodes. Water samples were transported under refrigeration to the laboratory, filtered through fiberglass filters and then analyzed for nutrients (NH_4–N, NO_3–N, NO_2–N and total phosphorus) according to APHA (1992).

2.2. Cyanobacteria identification and counts

Water samples for cyanobacteria analysis were preserved with Lugol's solution and species identified by light microscopy (Olympus). Cells counts were performed using a 1 ml Sedgwick-Rafter Cell and counting cells in at least 10 random fields. The cell numbers of species forming colonies (e.g., *Microcystis* and *Gomphosphaeria*) were estimated multiplying the cell numbers average of at least five squares (100 μ^2 of area) of a calibrated Whipple Reticle by the number of squares that superposed the colonies (Jardim et al., 2002).

2.3. Isolation and culture

Non-axenic cultures of *A. spiroides* were isolated from a bloom sample collected on 27 March by transferring single trichomes with a Pasteur pipette to successive drops of culture media and finally to a culture tube containing approximately 5 ml sterile ASM-1 medium (Gorham et al., 1964). The three strains (ITEP-024, ITEP-025 and ITEP-026) were maintained at 26 ± 2 °C with a photon flux density of 40 μmol photons m^{-2} s^{-1} (Biospherical Instruments QSL-100) from cool-white fluorescent tubes on a 12 h light cycle:12 h dark cycle.

To obtain sufficient biomass for the analyzes, the strains were cultivated in 2 l erlenmeyer flasks