Microcystins produced by filamentous cyanobacteria in urban lakes. A case study in Mexico City

Microcistinas producidas por cianobacterias filamentosas en lagos urbanos. Un estudio de caso en la Ciudad de México

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ABSTRACT

Cyanobacterial blooms are of great importance because of the toxic effects that these microorganisms are able to induce, particularly on aquatic organisms. Microcystins (MCs) are the principal toxins biosynthesized by cyanobacteria and are powerful inhibitors of the protein phosphatases 1 and 2A. Zooplankton filter feeders such as cladocerans are directly affected by MCs as a result of ingestion of cyanobacteria or contact with intracellular products when cyanobacterial cells break up during and after blooms. A total of 17 strains of filamentous cyanobacteria isolated from three urban lakes in Mexico City were characterized using the microcystin synthetase region *mcyA*-Cd. Acute 48-h toxicity was evaluated in different strains using the cladoceran *Daphnia magna* and total microcystin content was determined by enzyme-linked immunosorbent assay (ELISA). The *mcyA*-Cd region was amplified in 16 microcystin-producing strains; microcystins were detected in eight strains with values ranging from 0.1422 to 2.772 $\mu g\ L^{-1}$. Nevertheless, all aqueous crude extracts induced acute toxicity in *D. magna* with LC_{50} values from 363.91 to 741.8 mg L^{-1} (dry weight). The toxicity observed in non-microcystin-producing strains may be induced by cyclic peptides other than microcystins (anabaenopeptins, microviridins and cyclamides). The results obtained warn of the toxigenic potential of filamentous cyanobacteria, since though *Microcystis* spp. is frequently predominant in blooms, other toxins and intracellular metabolites released by filamentous cyanobacteria may induce toxicity on aquatic organisms as well as humans.

Key words: Acute toxicity, cyanotoxins, *Daphnia magna*, *mcyA*-Cd region, *Microcystis* ssp.

RESUMEN

Los florecimientos ("*blooms*") de cianobacterias son de gran importancia por los efectos tóxicos que estos microorganismos pueden desencadenar, principalmente en los organismos acuáticos. Las microcistinas (MCs) son las principales toxinas sintetizadas por las cianobacterias y son potentes inhibidores de las proteínas fosfatasas 1 y 2A. El zooplancton filtrador, como los cladóceros, es afectado por las microcistinas a través de la ingesta de células o filamentos cortos de cianobacterias o por el contacto con los productos intracelulares cuando las células se rompen después de un "*bloom*". Un total de 17 cepas de cianobacterias filamentosas aisladas de tres lagos urbanos de la Ciudad de México fueron caracterizadas usando la región *mcyA*-Cd de la microcistina sintetasa. Se evaluó la toxicidad aguda a las 48 h con el cladócero *Daphnia magna* y se determinó el contenido de microcistinas mediante ELISA. Se amplificó la región *mcyA*-Cd en dieciséis cepas productoras de microcistinas, aunque se detectaron microcistinas sólo en ocho,

obteniendo valores de 0.1422 a 2.772 μg L^{-1}. Sin embargo, todos los extractos crudos acuosos produjeron toxicidad aguda en *D. magna* (CL_{50} de 363.91 a 741.8 mg L^{-1}, biomasa seca). La toxicidad observada en las cepas no productoras de microcistinas pudo deberse a otros péptidos cíclicos diferentes a las microcistinas (anabaenopeptinas, microviridinas y ciclamidas). Los resultados obtenidos advierten del potencial toxigénico de las cianobacterias filamentosas, ya que a pesar de que *Microcystis* spp. predomina con frecuencia en los *blooms*, otras toxinas y metabolitos intracelulares liberados por cianobacterias filamentosas pueden ser tóxicos para organismos acuáticos, así como para los humanos.

Palabras clave: Cianotoxinas, *Daphnia magna*, *Microcystis* ssp., región *mcyA*-Cd, toxicidad agua.

INTRODUCTION

Formation of cyanobacterial blooms in water bodies with a high nutrient content (mostly P and N) is a frequent occurrence affecting freshwater quality at all latitudes (Babica *et al*., 2006b). Bloom development is of great importance since cyanobacteria biosynthesize a large number of secondary metabolites, and some of them are toxic to other organisms (Briand *et al*., 2008); according some reports microcystins (MCs) are the most abundant and well known cyanobacterial toxins (Pflugmacher & Wiegand, 2001).

MCs are biosynthesized by some 14 different cyanobacteria genera (Žegura *et al*., 2011). These toxins are potent inhibitors of the serine/threonine phosphatases 1 and 2A (PP1 and PP2A) (MacKintosh *et al*., 1990). Chemically, they are cyclic heptapeptides with general structure cyclo-(D-Ala^1-L-X^2-D-$MeAsp^3$-L-Z^4-Adda-Arg^5-D-Glu^6-$Mdha^7$-). They have five invariable amino acids: D-alanine (position 1), D-methyl aspartic acid (position 3), Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-dienoic acid, position 5), D-glutamic acid (position 6), and *N*-methyldihydroalanine (position 7); and two variable L-amino acids (X and Z) at positions 2 and 4 (Sivonen & Jones, 1999; Kaebernick & Neilan, 2001; Wiegand & Pflugmacher, 2005; Jayaraj *et al*., 2006).

MCs are assigned a name based on the two variable amino acids, the best known being MC-LR which contains the amino acids leucine (L) and arginine (R). Over 85 variants of MCs have been recognized and are identified by degree of methylation, peptide sequence and individual toxicity (Sivonen & Jones, 1999; del Campo *et al*., 2010). A single strain has also been shown to be able to biosynthesize more than one variant of these toxins (Sivonen & Jones, 1999; Kaebernick & Neilan, 2001; Wiegand & Pflugmacher, 2005; Jayaraj *et al*., 2006).

MCs can be identified and quantified by using analytical methods such as high-performance liquid chromatography (HPLC), mass spectrometry (MS) or matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) (Nicholson & Burch, 2001); immunoassay methods like enzyme-linked immunosorbent assay (ELISA) (Hawkins *et al*., 2003); by protein phosphatase inhibition (PP1 and PP2A) (Henning *et al*., 2001); or by molecular methods via amplification of one of the genes of the *mcy* cluster (Tillett *et al*., 2000; Kaebernick & Neilan, 2001). Their effects can also be evaluated by means of bioassays using mainly cladocerans, other types of crustaceans, gastropods, fish or mice (Rohrlack *et al*., 2001; de Figueiredo *et al*., 2004; Wiegand & Pflugmacher, 2005).

The biological or ecological role of MCs is not known for certain, but some of their hypothetical functions include: taking part in photosynthesis (since the greatest part of total intracellular MC content is located in the thylakoid membrane) (Young *et al*., 2005), in production of siderophores as chelators of metals (such as iron) (Utkilen & Gjolme, 1995); interacting in the regulation of gene expression (Kaebernick *et al*., 2002; Martin-Luna *et al*., 2006; Ginn *et al*., 2010) , and as an allelopathic substance (Pflugmacher, 2002; Sukenik *et al*., 2002; Babica *et al*., 2006b). Several studies have also referred to their role as a perceptible substance in the cyanobacterial membrane, perhaps helping to prevent ingestion of these microorganisms by herbivorous zooplankton (Kaebernick *et al*., 2000; Jang *et al*., 2003). In this regard, herbivorous zooplankton may be directly affected by the ingestion of cyanobacteria as part of their diet. Furthermore, like other organisms, they can bioaccumulate these toxins passing them up through the food chain (Ferrão-Filho *et al*., 2009; Prakash *et al*., 2009).

Microcystis is the main MC-producing genus (Sivonen, 1996) and most studies have focused on it. Limited information exists on other potentially toxigenic genera that are also able to produce cyanotoxins such as the filamentous cyanobacteria *Anabaena, Planktothrix, Cylindrospermopsis*, *Pseudanabaena*, *Oscillatoria* and *Nostoc*. In freshwater environments, MCs constitute a persistent severe hazard for ecosystem equilibrium and human health, since they can induce damage on aquatic biota and also contaminate both drinking and recreational water.

In Mexico, there have been several reports of occurrence of *Microcystis aeruginosa* (Kützing) Kützing, mixed with *M. botrys* Teiling and other cyanobacteria, in blooms occurring in tropical reservoirs in the State of Mexico (Gaytan-Herrera *et al*., 2011), as well as in Mexico City (Chapultepec, Alameda Oriente and Cuemanco) (Alcocer *et al*., 1988; Alva-Martínez *et al*., 2007; Kómarek & Komárková, 2002; Arzate-Cárdenas *et al*., 2010; Vasconcelos *et al*., 2010). Different cyanobacteria species have been documented in Quintana Roo, the State of Mexico, Veracruz and Mexico City (Vasconcelos *et al*., 2010). Despite these reports, there are few studies identifying the MC-producing strains, and their toxigenic potential has not been evaluated.

Since cyanobacterial bloom formation is frequently increased in water bodies with high nutrient content (eutrophication) and because of the absence of information on other cyanobacteria genera besides *Microcystis* potentially able to produce MCs, the present study has examined the toxicity of 17 filamentous cyanobacteria strains previously isolated from three urban lakes in Mexico City currently undergoing eutrophication. To this aim, molecular analysis of the strains was conducted using primers targeting the condensation domain of the microcystin synthetase gene *mcyA*. Also, in order to provide information on the toxigenic potential of filamentous cyanobacteria that are able to produce MCs or other nonspecific secondary metabolites, total MC content was determined by ELISA, and the toxigenic potential of individual strains was evaluated with acute toxicity bioassays using the cladoceran *Daphnia magna* Straus 1820.

MATERIALS AND METHODS

A total of 17 strains of filamentous cyanobacteria isolated in March 2007 from three urban lakes in Mexico City were selected for this study. The lakes are located at Chapultepec Park First Section (19º 25' 25.90" N, 99º 11' 5.03" W), Alameda Oriente Ecological Park (divided into five ponds, 19º 26' 13.09" - 19º 26' 8.36" N and 99º 3' 15.36" - 99º 3' 22.19" W) and the Olympic Rowing-Canoeing Course "Virgilio Uribe" at Cuemanco (19º 16' 20.58" N, 99º 6' 16.72" W) (Pineda-Mendoza *et al.*, 2011). These strains were taxonomically identified based on their morphological characteristics and the genetic marker 16S rRNA. In addition, the cyanobacterium *Anabaena flos-aquae* Brébisson ex Bornet & Flauhault (UTEX LB2358) was used as a reference strain in molecular identification. All strains were deposited in the strain collection of the Laboratorio de Hidrobiología Experimental at the Escuela Nacional de Ciencias Biológicas, IPN.

Strains were cultured in 500-mL Erlenmeyer flasks with liquid BG-11 mineral medium (Rippka, 1988), except for *Arthrospira* sp. which was grown in Zarrouk (1966) culture medium. Cultures were maintained under constant aeration, white light illumination (21 µmol photons m^{-2} s^{-1}), at 16:8 h (light: dark) photoperiod and 25 ± 1 °C.

To extract genomic DNA, 50 mg of 15-day biomass were used. Samples were frozen in liquid nitrogen and macerated until a fine powder was obtained. DNA extraction was subsequently made by the CTAB (cetyl trimethyl ammonium bromide) method described by Allers & Lichten (2000) and modified by Rodríguez-Tovar *et al.*, (2005). Samples were stored at –20 °C until further use.

The *mcyA*-Cd region was amplified using the primers *mcyA*-Cd 1F and *mcyA*-Cd 1R described by Hisbergues *et al.*, (2003). The reaction was carried out in a final volume of 25 µL which contained 50 ng DNA, 1X enzyme buffer, 2.5 mM $MgCl_2$, 200 µM of each of the dNTP, 10 pmol of each primer, 10 mg μL^{-1} bovine serum albumin (BSA) and 1.25 U *Taq* polymerase (Invitrogen, Mexico).

PCR reaction conditions were: initial denaturation cycle at 95 °C for 10 min; followed by 35 cycles at 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s; and final extension at 72 °C for 5 min.

PCR amplification products were run on a 1.5% agarose gel. A marker with molecular size 100 bp (Invitrogen) was used as a reference. The gels were stained with ethidium bromide (10 µg mL^{-1}, Sigma®) and visualized in a UV transilluminator (Alpha Innotech Imager 2000).

A total of 500 mL of 15-day culture grown from each of the strains was centrifuged at 2,191 x *g* for 30 min. The pellet was dried in an oven at 40 °C for 12 h. After the dry biomass was obtained, 300 mg was taken and ground with dry ice.

The ground sample was resuspended in 40 mL hard water and sonicated in 9.9 x 5 s pulses for 10 min at 25% amplitude and 4 °C, using an Ultrasonic Homogenizer sonicator (Cole-Parmer). The sample was then centrifuged at 2,191 x *g* for 1 h to eliminate any cellular remains.

The supernatant was removed and diluted to 50 mL in reconstituted hard water (USEPA, 2002), then passed through a 3 µm-pore-size nitrocellulose membrane. The filtered extract was made to attain a volume of 300 mL by adding reconstituted hard water, obtaining a final concentration of 1,000 mg L^{-1}.

Quantification of total MCs (MC-LR, -LA, -RR and -YR) was performed for each of the strains with ELISA, using the Quanti-Plate™ kit for MCs (Envirologix™) according to manufacturer's instructions. The crude extracts adjusted to 1 mg mL^{-1} dry biomass which were used in the acute toxicity bioassays on the cladoceran *Daphnia magna* were employed for MC quantification.

Enzyme conjugate concentration was measured at 450 nm, the best fit equation was obtained and the MC content of the sample was estimated by interpolation. MC content was expressed as MC-LR equivalents (µg L^{-1}). Quantification was performed in duplicate.

To conduct the bioassays, each lot was started with neonates obtained from the second brood on (age < 24 h) and from parthenogenetic females. A total of ten specimens were placed in 500-mL wide-mouth containers containing reconstituted hard water as a culture medium and fed with the microalga *Ankistrodesmus falcatus* (Corda) Ralfs at a concentration of 6 x 10^5 cells mL^{-1}. Food and culture medium were replaced three times a week. Lots were maintained in a bioclimatic chamber at 25 ± 1°C with at 16:8 h photoperiod.

Acute 48-h toxicity of the aqueous crude extracts obtained from each of the strains was evaluated on the cladoceran *Daphnia magna* Straus determining the median lethal concentration (LC_{50}) according to the OECD (2004) guideline, which uses test organism immobility as an endpoint.

The crude extract concentrations assayed were 200, 400, 600, 800 and 1000 mg L^{-1} dry biomass (obtained from a concentrated solution), plus a negative control (reconstituted hard water). The assays were performed in triplicate.

In the acute toxicity evaluations, dissolved oxygen concentration and pH were determined at the beginning and end of the test. LC_{50} was determined by the Probit method.

RESULTS

A total of 17 cyanobacteria strains that had been previously identified by partial 16S rRNA gene sequencing were examined (Table 1), clustering into 10 different genera (Pineda-Mendoza *et al*., 2011).

The *mcyA*-Cd region was amplified for 16 of the 17 strains assayed as well as the reference strain (*Anabaena flos-aquae* UTEX LB2358), obtaining a product ~300 bp in size. The strain AD13-Z, corresponding to the cyanobacterium *Arthrospira* sp., could not be amplified despite the fact that the procedure was repeated several times; however, the strain was not eliminated from the study. In the strain AC11 (*Spirulina subsalsa* Lighner), two PCR amplification products were found, only one of them corresponded to the expected 300 bp size (Fig. 1). Table 1 summarizes the results obtained.

Table 1 lists MC content in crude extracts as quantified by ELISA. MC production was found in eight of the 17 strains: AA07 (*Planktolyngbya* sp.), AA10 (*Planktolyngbya* sp.), AC11 (*Spirulina* sp.), AC12 (*Anabaenopsis* sp.), AD26 (*Pseudanabaena mucicola*

Table 1. Cyanobacteria assayed listed by study site, showing taxonomic assignment, amplification of the *mcyA*-Cd region, total microcystin content as determined by ELISA, and LC_{50} values (95% confidence intervals) in *Daphnia magna* neonates exposed to aqueous crude extracts.

Source	Strain	Taxonomic assignment	*mcyA*-Cd region	Total microcystin content (μg L^{-1})	LC_{50} (mg L^{-1} dry weight)
Reference	UTEX LB2358	*Anabaena flos-aquae* Brébisson ex Bornet *et* Flauhault 1886	+	ND	604.5 (551.5-655.5)
Chapultepec-First Section	CHM1	*Leptolyngbya tenerrima* (Kützing ex Hansgirg) Komárek 2001	+	ND	459.67 (211.59-699.82)
	CH04	*Geitlerinema carotinosum* (Geitler) Anagnostidis 1989	+	ND	513.78 (454.19-572.88)
	CH12	*Leptolyngbya boryana* (Gomont) Anagnostidis *et* Komárek 1988	+	ND	553.64 (496.98-615.33)
Alameda Oriente	AA07	*Planktolyngbya* sp.	+	0.45210514	587.1 (501.86-687.43)
	AA09	*Planktolyngbya* sp.	+	ND	532.65 (461.89-608.28)
	AA10	*Planktolyngbya* sp.	+	0.16116055	431.02 (369.52-489.78)
	AB02	*Limnothrix* sp.	+	ND	583.9 (516.43-655.39)
	AC11	*Spirulina* sp.	+	0.14223285	418.76 (348.22-484.14)
	AC12	*Anabaenopsis* sp.	+	0.23167042	628.52 (553.73-771.87)
	AD13	*Pseudanabaena mucicola* (Naumann *et* Huber-Pestalozzi) Schwabe 1964	+	ND	363.91 (272.71-444.92)
	AD17	*Phormidium pseudopristleyi* Anagnostidis *et* Komárek 1988	+	ND	542.05 (470.12-618.97)
	AD25	*Planktolyngbya* sp.	+	ND	417.78 (336.78-520.77)
	AD26	*Pseudanabaena mucicola*	+	0.3490	741.8 (642.61-880.12)
	AD13-Z	*Arthrospira* sp.	–	ND	519.88 (450.79-589)
Olympic Rowing-Canoeing Course	PO01	*Planktothrix agardhii* (Gomont) Anagnostidis *et* Komárek 1988	+	2.77198952	515.7 (465-562.5)
	PO05	*Pseudanabaena* sp.	+	0.26869402	696.95 (596.65-843.94)
	PO07	*Limnothrix redekei* (van Goor) M.E.Meffert 1988	+	0.22087305	423.5 (313.3-439.91)

ND: Not detected, indicates that the value obtained was outside the detection range of the ELISA kit used. **+**: successful and **-**: unsuccessful amplification of the *mcyA*-Cd region.

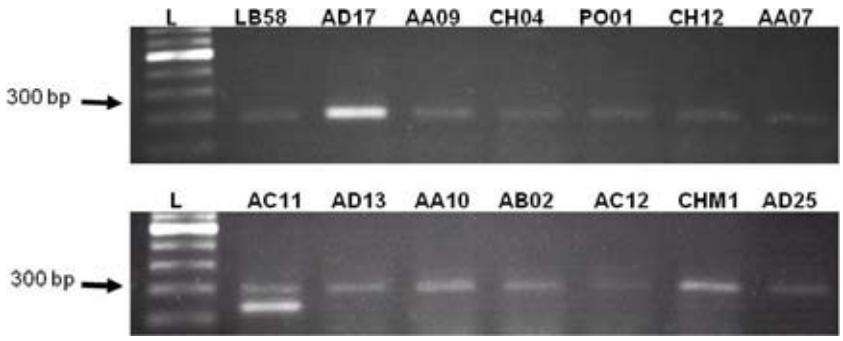

Figure 1. Amplification of the *mcyA*-Cd region. L: 100 bp DNA ladder; LB58: *Anabaena flos aquae* UTEX LB2358, positive control; other lanes represent the different strains in the study (see list in Table 1). These two gels demonstrate the presence of the gene A, which is part of the cluster responsible of MC's synthesis in these strains.

(Naumann *et* Huber-Pestalozzi) Schwabe), PO01 (*Planktothrix agardhii* (Gomont) Anagnostidis *et* Komárek), PO05 (*Pseudanabaena* sp.) and PO07 (*Limnothrix redekei* (van Goor) Meffert). The minimum content was found in the strain AC11 (0.1422 μg L^{-1}) and the maximum in PO01 (2.772 μg L^{-1}).

The means of the 48-h LC_{50} are shown in Table 1. Strains AD13 (*Pseudanabaena mucicola*) and AD25 (*Planktolyngbya* sp.) had the lowest LC_{50} values (363.91 and 417.78 mg L^{-1} dry biomass, respectively) and were the strains most toxic to *D. magna*. As can be seen in Table 1, although MCs were not detected in all of the strains, all of the strains induced acute toxicity, the highest LC_{50} corresponding to the strain AD26 (*P. mucicola*) which did not produce MCs. It should be noted that although the *mcyA*-Cd region was amplified successfully from the two strains most toxic to *D. magna*, these strains did not produce MCs according to the ELISA technique used, and the toxicity induced must therefore be due to other intracellular compounds present in the crude extract.

DISCUSSION

In Mexico City, there are various artificial lakes used for recreational and/or sport activities in which cyanobacterial bloom formation is frequently increased due to the high degree of eutrophication of these water bodies. This deserves special attention given the potential risk to human health and animal sanitation posed by blooms, since toxicity has been documented to occur in 25-60% of all cyanobacteria-containing samples (Chorus, 2001; Bláha *et al.*, 2009). More specifically, studies in several European and Asian countries found MCs in 60-90% of all the samples assayed (Chorus, 2001; Kotut *et al.*, 2006).

On the other hand, MC-producing strains coexist in blooms with strains in which this capacity is lacking (Harada *et al.*, 1999; Sheng *et al.*, 2006) and cannot be differentiated on the basis of morphological characteristics (Pearson & Neilan, 2008); therefore use of the *mcyA*–Cd region was of help in discerning the toxigenic potential of strains. This is supported by the fact that the presence of *mcy* genes is usually related to MC production and therefore the absence of such genes does not permit biosynthesis of these toxins to take place (Kurmayer *et al.*, 2002; Martins *et al.*, 2009). However, what actually determines MC biosynthesis are diverse environmental factors such as temperature, pH, light intensity, nutrient concentration (nitrogen, phosphorus and iron) and presence of filter feeders (Kaebernick & Neilan, 2001; Amé & Wunderlin, 2005; Almeida *et al.*, 2006), so that even when a strain has toxigenic potential, certain environmental conditions are required in order to allow expression of these genes.

ELISA permitted total MCs (MC-LR, MC-LA, MC-RR and MC-YR) to be quantified in some of the strains. The maximum MC value, 2.772 μg L^{-1} was found in strain PO01 (*Planktothrix agardhii* (Gomont) Anagnostidis *et* Komárek from the Olympic Rowing-Canoeing Course). The World Health Organization (WHO) indicates that the guideline for MC-LR in public drinking water systems is 1 μg L^{-1} (WHO, 1998). Although the value found here is higher than the accepted maximum, *P. agardhii* grows disperse without forming scums on the water body surface and usually no remedial measures are taken since the total cyanobacteria biomass is underestimated with this type of growth (Fastner *et al.*, 2003). On the other hand, since this cyanobacterium does not form scum, the risk to organisms within the affected environment as well as humans (in this case users of the rowing-canoeing course) may be lower, since cells of this cyanobacterium remain disperse and any potential MC releases would consequently become diluted within the water body (WHO, 2003).

However, it must be recalled that ingestion for prolonged periods of drinking water contaminated with MCs may lead to development of liver cancer (Bouaïcha, 2005) and users of recreational waters containing cyanobacteria have been shown to accidentally ingest 100 to 200 mL water (WHO, 2003). This is an important point for sanitation authorities to consider, it being necessary therefore to put up signs warning of the potential risk of water contamination by cyanobacteria.

MCs were detected and quantified in only some of the strains evaluated (*Planktolyngbya* sp., *Spirulina* sp., *Anabaenopsis* sp., *Pseudanabaena mucicola*, *Planktothrix agardhii*, *Leptolyngbya* sp. and *Limnothrix redekei* (van Goor) Meffert) while in other *Planktolyngbya* sp. isolates MC content quantification was possible in only two of the strains. This does not necessarily indicate that these cyanobacteria do not produce cyanotoxins, absence of the latter is more likely due to the fact that the ELISA method used recognizes only four isoforms of MCs (MC-LR, -LA, -RR and -YR) and some 85 different MCs have been reported to date (del Campo & Ouahid, 2010). This leaves as a possibility for future studies the species-specific identification of isoforms of other MCs, using analytical methods such as HPLC-MS.

On the other hand, evaluation of the toxic effects of MCs on cladocerans has been carried out by exposing test organisms to MC in a pure form, to crude extract from the strains isolated (the total cellular content of the cyanobacteria) or to crude extract of biomass obtained from blooms. Crude extracts have been found to be more toxic than pure MC, a fact that is explained as resulting from potential synergism between metabolites produced by the cyanobacteria strains assayed (Sedmak *et al.*, 2008b).

Sotero-Santos *et al.* (2006) exposed the cladocerans *Daphnia similis* Claus and *Ceriodaphnia silvestrii* Daday to crude extract of the cyanobacterium *Microcystis aeruginosa*, finding LC_{50} values of 186.61 and 155.11 mg L^{-1} dry biomass, respectively. Similarly, Okumura *et al.* (2007) assayed crude extracts from blooms formed in Barra Bonita and Ibitinga (São Paulo, Brazil) (both dominated by *Microcystis* spp.) using the cladocerans *D. similis*, *C. dubia* and *C. silvestrii*, and found LC_{50} values ranging from 32.6 to 80.2 µg MC g^{-1} dry material.

The LC_{50} values obtained in the present study are higher than those reported in the latter studies, ranging from 363.91 to 741.8 mg L^{-1} dry biomass. This suggests that the crude extracts obtained from the cyanobacteria assayed in our study were less toxic. However, it should be remembered that our data refer exclusively to the effects of cyanotoxins (and other metabolites) produced by filamentous cyanobacteria, while *Microcystis* is the primary MC-producing taxon (Martins *et al.*, 2009) and therefore most toxicity studies refer to this genus. Furthermore, there are few studies available on the toxic effects of filamentous cyanobacteria and consequently the present study provides knowledge on the toxicity induced by cyanobacteria other than *Microcystis* that were capable of inducing adverse effects on cladocerans.

In contrast, in the strain AD13-Z (*Arthrospira* sp.) biosynthesis of MCs was not detected by any of the methods used, although in the acute bioassay using *D. magna* toxic effects were observed. This may be explained as a result of cyanobacteria being able to biosynthesize other metabolites which elicit harmful effects on aquatic organisms (Sivonen & Jones, 1999).

A different situation was observed in strains CH04, AA09, AB02, AD13 and AD17, in which the *mcyA*-Cd region was successfully amplified and although MC biosynthesis was not confirmed by ELISA, toxic effects were found to occur in daphniids. This confirms that the toxicity observed is due not only to MCs but is also perhaps induced by other cyclic peptides such as cyanopectolins, aeruginosins, anabaenopeptins, microviridins and cyclamides, among other molecules (Welker *et al.*, 2006). Despite the fact that these molecules are said to be nontoxic, all these compounds inhibit protease activity (Sedmak *et al.*, 2008a) and have been found in diverse tissues of aquatic organisms (Sedmak *et al.*, 2008b).

The toxicity of extracts from strains in which the *mcyA*-Cd region was not detected or in which MCs were not quantified may also be due to synergistic interactions between different toxic and "nontoxic" metabolic products of the cyanobacteria contained in the crude extracts of the strains assayed. This has been documented in diverse studies in which exposure to crude cyanobacteria extracts induced drastically higher toxic effects in vertebrates and invertebrates than exposure to the pure form of the toxins (Keil *et al.*, 2002; Šuput *et al.*, 2002; Sedmak *et al.*, 2008b).

It is worth noting that variation was found in the LC_{50} values of strains AA07, AA09, AA10 and AD25, corresponding to the genus *Planktolyngbya* sp. This can be explained in terms of existing differences in toxin production among genetically similar strains which elicits variations in their toxigenic potential (Thacker & Paul, 2004).

In conclusion, based on results obtained in the acute assay using cladocerans, the strains with the highest acute toxicity were *Pseudanabaena mucicola* (AD13), *Plaktolyngbya* sp. (AD25, AA10) and *Spirulina* sp. (AC11). Also, the analytical methods used in the present study (PCR and ELISA) were useful and reliable for determining MC production and its potential contribution to the acute toxic effects observed in the daphniids.

Last of all, the present study provides important information on detection of MCs in filamentous cyanobacteria that colonize urban lakes, and constitutes the basis for the early detection of strains of toxigenic cyanobacteria, which is essential to prevent potential damage to both human health and aquatic biota, allowing the implementation of preventive or remedial actions. It is also worth noting that although most cyanobacterial blooms are caused by the genus *Microcystis*, attention must be paid to other types of these organisms, such as filamentous cyanobacteria, as they may contribute to the toxic effects observed in aquatic organisms and potentially also humans.

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