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Infochemicals released by *Daphnia magna* fed on *Microcystis aeruginosa* affect *mcyA* gene expression

Rosa María Pineda-Mendoza [a,b], Gerardo Zúñiga [b], Fernando Martínez-Jerónimo [a,*]

[a] Laboratorio de Hidrobiología Experimental, Departamento de Zoología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prol. Carpio esq. Plan de Ayala s/n, Col. Sto. Tomás, Mexico, D.F. 11340, Mexico
[b] Laboratorio de Variación Biológica y Evolución, Departamento de Zoología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prol. Carpio esq. Plan de Ayala s/n, Col. Sto. Tomás, Mexico, D.F. 11340, Mexico

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ABSTRACT

Microcystins (MCs) are toxic heptapeptides produced by cyanobacteria during blooms that are noxious to diverse organisms, from bacteria to vertebrates. Specifically in daphnids, they cause reduced growth, a low reproductive rate, and, in extreme cases, death; however, different infochemicals released by cladocerans stimulate MCs synthesis. Ecological cyanobacteria–daphnids interactions are complex and not clear yet. In this study, we evaluated the effects of infochemicals released by *Daphnia magna* neonates and adults fed with different concentrations of *Microcystis aeruginosa* on population growth of strains Ch10 and UTEX LB2385 of *M. aeruginosa*, *mcyA* gene expression in real time qPCR, and the intracellular concentration of MCs. In addition, we assessed the relation between the cellular diameter and the intracellular concentration of MCs in both strains. Chlorophyll content per cell was affected by the presence of infochemicals from *D. magna* neonates and adults. *mcyA* gene was significantly overexpressed in the early stages of population growth (5 days) in all treatments with strain UTEX LB2385, whereas overexpression was observed in strain Ch10 at the end stage of the exponential and stationary phases (10 and 15 days). Intracellular concentration of MCs varied with the tested factor. Results suggest that the increase in *mcyA* gene expression and in MCs production could be defense mechanisms against the consumption by *D. magna*. Results also demonstrate the physiological plasticity among *Microcystis* strains, which could explain the permanence and dominance of this genus in toxic blooms.

1. Introduction

During toxic blooms, different strains of the genus *Microcystis* grow predominantly and produce secondary metabolites like microcystins (MCs) (Carmichael, 1992; Chorus and Bartram, 1999; Pflugmacher et al., 1999; Sivonen and Jones, 1999), which are potent inhibitors of eukaryotes protein-phosphatases (Ppp1, Ppp2, Ppp4, Ppp5, and Ppp6) (Carmichael et al., 2001; Dittmann and Wiegand, 2006; Pereira et al., 2010; Moreira et al., 2013).

The toxic effects of MCs have been evaluated in organisms such as bacteria, fungi, microalgae, invertebrates, fishes, amphibians, birds, and mammals (Duy et al., 2000; Vasconcelos, 2001; Briand et al., 2003; Chen et al., 2009). Specifically in cladocerans, different *Daphnia* species have been used to evaluate the toxic and nutritional effects of *Microcystis* (DeMott, 1999; Ferrão-Filho et al., 2000; Rohrlack et al., 2001; Lürling, 2003; Lürling and van der Grinten, 2003; Okumura et al., 2007; Dao et al., 2010), as

* Corresponding author. Tel.: +52 55 5729 6000x62424; fax: +52 55 5729 6000x46211.
E-mail addresses: ferjeronimo@hotmail.com, fjeroni@ipn.mx (F. Martínez-Jerónimo).

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well as to know the ecological cyanobacteria–daphnids interactions (Jang et al., 2003; van Gremberghe et al., 2009).

In this sense, it has been observed that diverse characteristics of cyanobacteria limit their consumption by the zooplankton. Among them are restrictions in ingestion due to their morphological characteristics (colonial aggregation, thick filaments, and production of mucilage or sheaths) (Fulton and Pearl, 1987; de Bernardi and Giussani, 1990), deficiency in nutritional constituents (mainly lack of essential fatty acids) (von Elert and Wolffrom, 2001), and most importantly, their toxicity (Lampert, 1982; de Bernardi and Giussani, 1990).

Toxigenic strains of *Microcystis aeruginosa* inhibit strongly their use as food for daphnids since they reduce their growth, induce a low reproduction rate, and, in general, bring about a reduction in the population size (DeMott and Dwahale, 1995). On the other side, it has been reported that chemical signals (infochemicals) released by predators induce defenses in their preys (Bronmark and Hansson, 2000), as occurs with the zooplankton in the presence of fish (Loose et al., 1993). It has also been stated that this type of chemical communication occurs between the phytoplankton and the zooplankton (Larsson and Dodson, 1993; Jang et al., 2003). In this regard, filter-feeding daphnids that consume phytoplankton release infochemicals that induce morphological changes in the phytoplankton (e.g., *Scenedesmus* and *Desmodesmus*) (Lürling and Van Donk, 1997; van Gremberghe et al., 2009); specifically, in *Microcystis* species, they promote colony formation, reduced growth rate, and increased synthesis of MCs; thus, it has been proposed that this strategy could be a defense mechanism against foraging by the herbivorous zooplankton (Jang et al., 2003, 2008; Lürling and Van Donk, 1997; Becker, 2010).

Notwithstanding, the stress produced by the filter-feeding zooplankton has been discarded by some authors as the main cause of increment in the synthesis of MCs (Rantala et al., 2004; Becker, 2010). The results observed in other studies have demonstrated that the presence of bioactive substances released by planktonic daphnids (e. gr. infochemicals) can increase microcystins concentration (Yang et al., 2006; Jang et al., 2007, 2008; van Gremberghe et al., 2009), resulting from stimulation of the microcystin-synthetase genes expression. In order to test this hypothesis, in the present study, we evaluated the effects exerted by the infochemicals released by *Daphnia magna* neonates and adults fed with *M. aeruginosa* on: a) population growth of strains UTEX LB2385 and Ch10 of *M. aeruginosa*, b) the expression pattern of *mcyA* gene by RT-qPCR (gene of the microcystin synthetase cluster), and c) intracellular concentration of MCs in crude extracts. In addition, we determined the correlation between the intracellular MCs content and the cellular size of the two assessed *M. aeruginosa* strains.

2. Material and methods

2.1. Test organisms and culture conditions

Two *M. aeruginosa* strains that produce MCs (Arzate-Cárdenas et al., 2010) were used: reference strain UTEX LB2385 (isolated from the Little Rideau Lake, Ontario, Canada), and the wild strain Ch10 isolated from the Lago Menor of the Chapultepec Park (urban lake), in Mexico City. The geographic origin of both strains enables a comparison that could be interesting in terms of the possibility of differences in their responses to the same experimental conditions.

Both strains were cultured in 150 mL Erlenmeyer flasks containing liquid mineral medium Z8 (5 mM $NaNO_3$, 0.18 mM K_2HPO_4, 0.1 mM $MgSO_4$, 0.25 mM $CaCl_2$, 0.2 mM Na_2CO_3, 0.01 mM disodium EDTA, 0.01 mM $FeCl_3$, and a micronutrients solution, at pH 10) (Kotai, 1972). All cultures were kept in an environmental chamber at 25 ± 1 °C with constant aeration, illuminated with "daylight" fluorescent lamps at 25 μmol of photons m^{-2} s^{-1}, and a 16:8 (light:-dark) photoperiod.

The zooplankton cladoceran *D. magna* was cultured in 500-mL containers using reconstituted hard water (206 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 247 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 193 mg L^{-1} $NaHCO_3$, and 8 mg L^{-1} KCl; hardness 160–180 mg L^{-1} as $CaCO_3$) (US EPA, 2002) as culture medium, it was fed with *Ankistrodesmus falcatus* at a concentration of 3×10^5 cells mL^{-1}, maintained in an environmental chamber at 25 ± 1 °C and a 16:8 h photoperiod.

2.2. Total DNA extraction, amplification and sequencing of the mcyA-*Cd region*

Total DNA extraction and amplification of the *mcyA*-Cd region was performed according to Pineda-Mendoza et al. (2012). Amplification of the *mcyA*-Cd region was performed using the primers reported by Hisbergues et al. (2003). Amplicons were sequenced using primer *mcyA*-Cd 1F. Identity of amplicons was confirmed by means of BLASTn (www.ncbi.nlm.nih.gov). Sequences were deposited in GenBank under access numbers KF372573 (strain Ch10) and KF372574 (strain UTEX LB2385).

From the sequences obtained from the *mcyA*-Cd region, the primers and the corresponding hydrolysis probe (Applied Biosystems, Foster City, CA, USA) were designed and deposited in the Probe database of NCBI. The fragment of the target gene is located between position 3621 and 3735 bp of the *mcyA* gene of *M. aeruginosa* NIES – 843 (access number: NC_010296.1). The intergenic space region of the phycocyanin operon (PC) was used as reference gene (Kurmayer and Kutzenberger, 2003).

2.3. Bioinformatics analysis of the secondary structure of region mcyA-*Cd*

To assess if the secondary structure of *mcyA*-Cd region interfered with primers displacement in the RT-qPCR we predicted the secondary structure of single-stranded DNA with the MFOLD v. 3.1 software (http://www.bioinfo.rpi.edu/applications/mfold) based on the minimal free energy and using the predetermined parameters and an aligning temperature of 60 °C. Tests were performed with 0 mM Na^+ and 25 or 50 mM Mg^{2+}, based on the concentrations reported in the *Taq* polymerase buffer (Invitrogen) used in the end point PCR and in the cDNA synthesis kit (Applied Biosystems), respectively.