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Effects of the *Microcystis aeruginosa* Strain RST9501 from Patos Lagoon, RS, on Growth and Reproduction of the Cladocera *Ceriodaphnia dubia*

N. J. C. Monteiro,[1*] J. S. Yunes[2] & M. B. Bohrer-Morel[3]

[1]Laboratório de Ecotoxicologia, CENECO, UFRGS, Porto Alegre, Brazil

[2]Unidade de Pesquisas em Cianobactérias, FURG, Rio Grande, RS, Brazil

[3]Laboratório de Ecologia e Ecotoxicologia Aquática, IPEN, USP, São Paulo, SP, Brazil

ABSTRACT

This study evaluates the toxicity of the *Microcystis aeruginosa* strain RST9501, isolated from Patos Lagoon, to cladocerans using standard tests with *Ceriodaphnia dubia*. The strain RST9501 was compared with the non-toxic *M. aeruginosa* strain NPJT-01. Chronic effects were estimated by evaluating reproduction and growth in *C. dubia* exposed to increasing concentrations of *M. aeruginosa*. Alternative food source constituted by *Pseudokirchneriella subcapitata* and *Artemia* chow was made available for all treatments. The presence of the two strains of *M. aeruginosa* inhibited both reproduction and growth in *C. dubia*. Also, regardless the presence of microcystin, there was an increase in toxicity as the cell concentration of both strains increased.

Key words: *Microcystis*, microcystin, *Ceriodaphnia*, toxicity, Brazil.

RESUMO

Estudos da toxicidade da cepa de *Microcystis aeruginosa* RST9501 da Lagoa dos Patos, RS, sobre *Ceriodaphnia dubia*

Este estudo avalia a toxicidade da cepa RST9501 de *Microcystis aeruginosa*, isolada da Lagoa dos Patos, para Cladocera, através de testes padronizados com *Ceriodaphnia dubia*. Para comparação de seus efeitos foi utilizada a cepa de *M. aeruginosa* NPJT-01 não tóxica para rato. Foi estimado efeito crônico a partir da avaliação da reprodução e crescimento de *C. dubia* em concentrações crescentes de *M. aeruginosa*, sendo que para todos os tratamentos foi disponibilizado alimento alternativo à base de *Pseudokirchneriella subcapitata* mais ração de artêmia. A presença de ambas as cepas de *M. aeruginosa* foi capaz de inibir a reprodução e o crescimento de *C. dubia*, e este efeito foi incrementado com o aumento na concentração de células de ambas as cepas, independente da presença de microcistina.

Palavras-cahve: *Microcystis*, microcistina, *Ceriodaphnia*, toxicidade, Brasil.

INTRODUCTION

The production of cyanobacterial toxins in aquatic environments can be extremely harmful to the plankton and to some fish species, and it is associated with a reduction on local diversity of both phytoplanktonic and zooplanktonic populations (Lampert, 1981; Codd, 1985; Paerl, 1988). Consequently, this impact will cause modifications in the behavior, composition and structure of the communities (Fulton & Paerl, 1987; Lampert, 1987; Forsyth *et al.*, 1990; Demott *et al.*, 1991; Jungmann, 1992; Reinikainen, 1994; Berthon & Brousse, 1995). Despite their low nutritional content, when low concentrations of cyanobacteria are present the inhibition of the filtering rates and the death of individuals of different species may suggest and point out to the high toxicity of certain strains (Nizan *et al.*, 1986; Hanazato & Yasuno, 1987; Gilbert, 1990; Demott *et al.*, 1991).

The aim of this study was to examine how a specific strain of *M. aeruginosa* (RST9501) microcystin containing, isolated in 1995 during investigations in Patos Lagoon (Yunes, 1996),

*Corresponding author: Nade Janara Coimbra, e-mail: nadejanara@ecologia.ufrgs.br.

affect *Ceriodaphnia dubia* growth and reproduction. We hypothesized that growth and reproduction will be only adversely affected when Microcystis microcystin containing is present. To test this hypothesis we mixed the green alga *Pseudokirchneriella subcapitata* with a microcytin-free and a strain of *M. aeruginosa* microcystin containing.

MATERIAL AND METHODS

The strain *M. aeruginosa* RST9501 used in this study was sampled and isolated from the Patos Lagoon estuary, and has been kept under culture conditions in the Cyanobacteria Research Unit, University of Rio Grande, Brazil, since 1995. The strain is well known by its content of 1D-leu MCYST, MCYST-FR, MCYST-LR (Matthiensen, 1999; Matthiensen *et al*., 2000).

The strain NPJT-01, isolated from the Juturnaíba Dam, Rio de Janeiro, in 1996, and kept in stock conditions in the Laboratory of Ecophysiology and Toxicology of Cyanobacteria (NPPN/UFRJ), is a non-toxic strain to mice and was used to compare the effects when there is no detectable production of microcystin.

Both strains of cyanobacteria were cultured in BGN1/2 medium (Rippka *et al.*, 1979), at 25°C, with constant aeration, photoperiod of 16h/8h (light:darkness) and light intensity of 125 $\mu^3 m^{-2}.s^{-1.}$

The evaluation of toxins content was performed by using an immunoassay, with the microcystin determination kit "EnviroGard Microcystins Plate Kit®" of Strategic Diagnostics Inc, USA. The toxins concentration of the strain RST9501 was determined for different cultures during the entire study period and for all cultures that were used in the toxicity tests. Only two dosages were done for the strain NPJT-01. The detection limit was ≥ 0.1 ppb.

C. dubia were exposed in a static renewal system to different concentrations during a seven-day period. The results were based on survival, growth and reproduction according to the short-term methods for measuring chronic toxicity of effluents and receiving waters to freshwater organisms – method 1002.0 of the U.S. Environmental Protection Agency (USEPA, 2002).

Individual organisms were exposed to 30 mL of test solutions at concentrations of 3×10^5, 6×10^5, 1.2×10^6, 2.5×10^6, 5×10^6, and 1×10^7 cells.mL^{-1} of *M. aeruginosa* and *Pseudokirchneriella subcapitata*. The control and test dilution water used was from a natural well, adjusted to pH 7.2 (± 2), and hardness ranging from 40 to 48 mg.L^{-1} of $CaCO_3$.

Growth was evaluated by measuring the total body length of three individuals daily for the entire period of the test. For this purpose, a stereoscopic microscope was used with a micrometered ocular connected to it. The statistic analysis of growth was performed by comparing the means of the differences between final length, taken in the 7th day, and initial length of each individual. The results were analyzed using the Bonferroni's t test (USEPA, 2002).

The reproduction data, represented by the offspring number per female, were analyzed using the Dunnett's multiple comparison test when data were normal and homogeneous, and using Steel's test when they were non-parametric. The normality was verified with the Chi-square and Shapiro-Wilks' tests and the homogeneity of the variance with the Hartley and Bartlett's tests. The Fisher's test was used to evaluate the toxic effects upon the survivorship of the organisms (USEPA, 2002).

RESULTS

The immunoassay analysis results that strain NPJT-01 contains no detectable microcystin, whereas RST9501 presents a microcystin content of 0.56 μg.mL^{-1} (2.75 μg.mg of dry weight^{-1}) with standard deviation 0.14 and coefficient of variation of 24%.

A significant increase in mortality was observed for *C. dubia* treated with microcystin containing strain (RST9501) and microcystin free strain (NPJT-01) at the concentrations of 1×10^7 cells.mL^{-1}, 5×10^6 cells.mL^{-1}, and 2.5×10^6 cells.mL^{-1} ($p = 0.05$). When reared with *Pseudokirchneriella subcapitata* alone, there was no mortality effect in *C. dubia*.

The presence of both strains of *M. aeruginosa* (toxic and non-toxic) caused a reduction in somatic growth in *C. dubia*. Body length at the end of the experiment was significantly lower as the concentration of cyanobacteria cells increased ($p = 0.05$).

In the treatments fed with microcystin containing RST9501, the difference was significant from the first concentration, 3×10^5 cells.mL^{-1}, with a 17% reduction in growth in comparison with control animals. At the concentrations of 6×10^5 cells.mL^{-1}, 1.2×10^6 cells.mL^{-1}, 2.5×10^6 cells.mL^{-1}, 5×10^6 cells.mL^{-1}, the reduction in somatic growth was 32, 38, 50, and 58%, respectively. For the highest evaluated concentration of this strain, 1×10^7 cells.mL^{-1}, the reduction in mean somatic growth was 70%.

Strain NPJT-01 at 3×10^5 cells.mL^{-1} increased body length in 7% when compared with the control. A significant reduction in body length was verified from 1.2×10^6 cells.mL^{-1} (31%) ($p = 0.05$). At 2.5×10^6 cells.mL^{-1} body length was 41% reduced, and at 5×10^6 cells.mL^{-1} it was 32% reduced. The maximum reduction in growth obtained with microcystin free NPJT-01 was 51% at 1×10^7 cells.mL^{-1}. When the same test was carried out with *Pseudokirchneriella subcapitata*, none of the concentrations evaluated significantly affected somatic growth. The maximum reduction observed was 12% (Figure 1).

The presence of both strains of *M. aeruginosa* promoted a statistically significant reduction ($p = 0.05$) in *C. dubia* reproduction (Table 1). A decrease in the offspring number was observed as the concentrations of cyanobacteria increased. When the cladocerans were exposed to the same concentrations of *Pseudokirchneriella subcapitata*, the reproduction was not significantly altered in comparison to the control group ($p = 0.05$). However, there was a tendency to stimulating *C. dubia* reproduction with increasing cell concentrations of *Pseudokirchneriella subcapitata*.

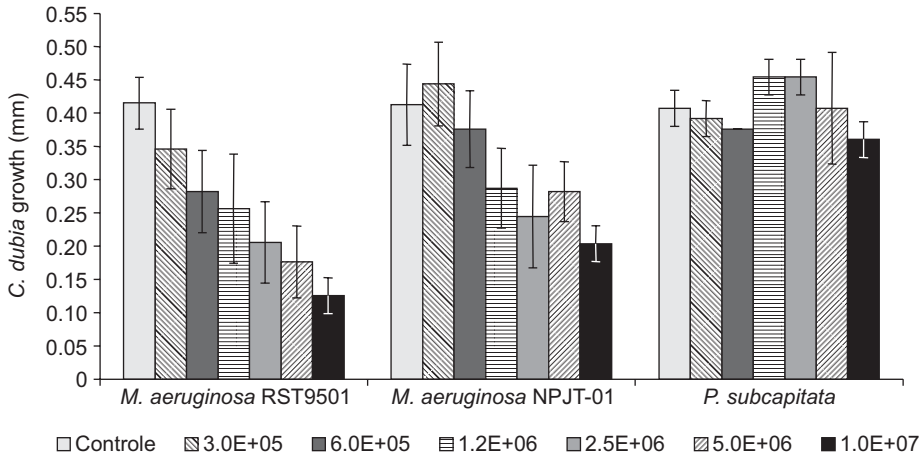

Figure 1 — Difference in size (growth) between the seventh and the first day in *C. dubia* exposed to *Microcystis* toxic strain (RST9501), non-toxic strain (NPJT-01) and *Pseudokirchneriella subcapitata*. Error bars indicate standard deviation.

Table 1 — Statistical results of the *C. dubia* reproduction, comparing the two strains of *Microcystis* (RST9501 and NPJT-01) in different concentrations.

Treatment	*Microcystis aeruginosa*				*Pseudokirchneriella subcapitata*	
	Strain RST9501		Strain NPJT01			
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Control	22.85^{ab}	6.79	26.15^{a}	7.87	20.30^{ab}	6.02
3.0E+05	18.03^{b}	11.19	22.80^{ab}	7.04	25.50^{a}	9.34
6.0E+05	13.39^{bc}	10.84	17.63^{b}	10.54	24.20^{abc}	3.49
1.2E+06	8.94^{c}	10.09	13.95^{bc}	9.01	27.00^{a}	5.46
2.5E+06	4.01^{cd}	5.78	6.83^{c}	6.73	24.70^{a}	4.45
5.0E+06	1.78^{d}	2.71	3.10^{cd}	3.81	32.10^{a}	9.18
1.0E+07	0.15^{e}	0.58	0.63^{de}	1.48	28.30^{a}	4.45

a. For the mean values, the letters a, b, c, d, and e indicate distinct significant differences in the analysis of variance complemented by the Dunnett multiple comparison test T3 (p = 0.05).

Comparing the two strains of *Microcystis* (RST9501 and NPJT-01), the effect upon *C. dubia* reproduction was not significantly different (p = 0.05) (Table 1). When *M. aeruginosa* or *Pseudokirchneriella subcapitata* were utilized as the only food source, a significant difference (p = 0.05) in offspring production was observed.

DISCUSSION

Toxin analysis has confirmed the presence of microcystins in RST9501 strain only. The concentration of this strain (1.64 μg.mg of dry weight^{-1}) is well within the range of 0.161 to 4.90 μg.mg of dry weight^{-1} reported in the literature for different strains (Reinikainen *et al*., 1994; Yunes *et al*., 1996; Matthiensen *et al*., 1999; Ferrão-Filho *et al*., 2000; Lürling & Van Der Grinten, 2003).

Survival, growth and reproduction of *C. dubia* were significantly reduced when both strains, microcystin containing (RST9501) and microcystin free (NPJT-01), were offered as food. *M. aeruginosa* have been reported to exert strong effects on Cladocera (Lampert, 1981; Fulton & Paerl, 1987; Hanazato & Yasuno, 1987; Gilbert, 1990; Lürling & Van Der Grinten, 2003). Both, increase in growth and delay in reproduction have been reported as chronic effects of *M. aeruginosa* in *Daphnia* (Reinikainen *et al.*, 1999).

The importance of the presence of microcystins for the zooplankton has been widely discussed (Nizan *et al.*, 1986; DeMott *et al.*, 1991; Jungemann, 1992; Lürling & Van Der Grinten, 2003; Reinikainen *et al.*, 1994; Rohrlack *et al.*, 1999a; Rohrlack *et al.*, 1999b). Contrasting with our results, mortality in *Daphnia* has been reported only when microcystins containing cells were present, suggesting microcystin-LR to be the likely cause of toxicity (Rohrlack *et al.*, 1999b).

Nevertheless, according to Reinikainen *et al.* (1994) and Lürling & Van Der Grinten (2003), *M. aeruginosa* toxicity to daphnids could be attributed not only to the microcystin, but also to the possible occurrence of other potentially toxic substances in the algae cells. Lethal effect was observed on *D. pulicaria* when a fraction of *M. aeruginosa* microcystins – free extract was used (Jungmann, 1992). Thus, it was suggested that a highly toxic component, different from microcystin-LR, could have been the cause of the toxicity.

Our findings suggest that a dose-related reduction on survival, growth and reproduction in *C. dubia* exposed to Microcystis may be a result of its low nutritional content. This is reinforced by the fact that there was no difference between the two analyzed strains (with or without microcystin) in terms of toxic effects. Cyanobacteria may be poorly digested or assimilated. In addition, it may lack essential unsaturated fat and other essential nutrients for the development of *C. dubia* (Lampert, 1987; DeMott, 1998; DeMott & Müller-Navarra, 1997).

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