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

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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.

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; Reiniakinen, 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),
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 microcystin-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, has been kept under culture conditions in the Cyanobacteria 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 µm–2.s–1.

The presence of both strains of cyanobacteria - M. aeruginosa and Pseudokirchneriella subcapitata - was sampled and isolated from the Patos Lagoon estuary, and a strain of M. aeruginosa microcystin containing.

RESULTS

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-Wilk’s 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).

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 × 107 cells.mL–1, 5 × 106 cells.mL–1, and 2.5 × 106 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 × 104 cells.mL–1, with a 17% reduction in growth in comparison with control animals. At the concentrations of 6 × 105 cells.mL–1, 1.2 × 106 cells.mL–1, 2.5 × 106 cells.mL–1, 5 × 106 cells.mL–1, the reduction in somatic growth was 32, 38, 50, and 58%, respectively. For the highest evaluated concentration of this strain, 1 × 107 cells.mL–1, the reduction in mean somatic growth was 70%.

Strain NPJT-01 at 3 × 105 cells.mL–1 increased body length in 7% when compared with the control. A significant reduction in body length was verified from 1.2 × 105 cells.mL–1 (31%) (p = 0.05). At 2.5 × 105 cells.mL–1 body length was 41% reduced, and at 5 × 105 cells.mL–1 it was 32% reduced. The maximum reduction in growth obtained with microcystin free NPJT-01 was 51% at 1 × 105 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 reproduction 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.
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**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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Microcystis aeruginosa</em></th>
<th><em>Pseudokirchneriella subcapitata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain RST9501</td>
<td>Strain NPJT01</td>
</tr>
<tr>
<td>Mean</td>
<td>Standard deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>Control</td>
<td>22.85(^{ab})</td>
<td>6.79</td>
</tr>
<tr>
<td>3.0E+05</td>
<td>18.03(^{b})</td>
<td>11.19</td>
</tr>
<tr>
<td>6.0E+05</td>
<td>13.39(^{bc})</td>
<td>10.84</td>
</tr>
<tr>
<td>1.2E+06</td>
<td>8.94(^{c})</td>
<td>10.09</td>
</tr>
<tr>
<td>2.5E+06</td>
<td>4.01(^{cd})</td>
<td>5.78</td>
</tr>
<tr>
<td>5.0E+06</td>
<td>1.78(^{d})</td>
<td>2.71</td>
</tr>
<tr>
<td>1.0E+07</td>
<td>0.15(^{e})</td>
<td>0.58</td>
</tr>
</tbody>
</table>

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 \(\mu\)g mg of dry weight\(^{-1}\)) is well within the range of 0.161 to 4.90 \(\mu\)g 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 microcysts – free extract was used (Jungemann, 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).

REFERENCES


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