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Highlighted article

Biodegradation of microcystins by aquatic *Burkholderia* sp. from a South Brazilian coastal lagoon

Gilmar A.F. Lemes[a], Ralf Kersanach[a], Luciano da S. Pinto[b], Odir A. Dellagostin[b], João S. Yunes[a], Alexandre Matthiensen[a,*]

[a]*Fundação Universidade Federal do Rio Grande (FURG), Av. Itália Km 08, 96201-900 Rio Grande-RS, Brazil*
[b]*Universidade Federal de Pelotas (UFPel), Campus Universitário, s/n, 96010-900 Pelotas-RS, Brazil*

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Abstract

Biological degradation of cyanobacterial hepatotoxin microcystins in estuarine and coastal water samples from the Patos Lagoon estuarine system, a coastal lagoon situated at the southernmost region of Brazil, was observed. Samples of natural surface water were spiked with purified and semi-purified microcystins (MC–LR and [D-Leu[1]]MC–LR) and their concentrations were monitored by HPLC analysis. After 15 days, the toxins were no longer detectable and after 43 days less than 90% of the initial concentration added to the samples was detected by ELISA. The average degradation rates and the exponential decay rate constants from inside and outside of the estuary were similar. A microcystin degradative bacterium was isolated from the estuarine region. Partial sequence of the 16S rDNA showed a 96% homology with the *Burkholderia* genus. This genus belongs to the beta subdivision on proteobacteria. This is the first report showing the genus *Burkholderia* as a cyanobacterial toxin degrader.

Keywords: Bacteria; Biodegradation; *Burkholderia*; Cyanobacteria; Microcystins; *Microcystis*; Patos Lagoon

1. Introduction

The Patos Lagoon is a coastal lagoon situated in the very south of Brazil (30°15′S and 52°10′W). It comprises a water body of approximately 10,360 km^2, and receives water from a drainage basin of almost 200,000 km^2, ending in a narrowed estuary system between the cities of Rio Grande and São José do Norte (Fig. 1). Despite its extensive area, it is a relatively shallow lagoon (average 4.2 m depth) and the water flow is generally lower in summer and autumn ($<1000\,m^3\,s^{-1}$) and higher in winter and spring ($>3000\,m^3\,s^{-1}$) (Herz, 1977).

The estuarine region represents approximately one-tenth of the total area. The dynamic action of wind patterns, plus other factors such as rainy or dry seasons, confers great salinity variability to the estuarine region. Temperature variations follow seasonal changes and range generally between 10 and 25 °C (Matthiensen, 1996). Since the drainage basin of the Patos Lagoon is very extensive, nutrient input to the lagoon and consequently to its estuary comes mainly from continental drainage. Regarding the phytoplankton community, in the estuarine region there is a dominance of a few diatom genera (Bergesch, 1990), but cyanobacteria may constitute the most abundant group in number of species and the formation and development of cyanobacterial blooms is frequently observed in summer.

The cyanobacteria group have gained crescent world-wide attention since the reports of cyanobacterial toxin (cyanotoxin) production by some species (Carmichael et al., 1985). Due to the high density of cells in bloom events the cyanotoxins may pose serious health risks to the biota and local human population. Toxic cyanobacterial blooms have been reported for Patos Lagoon in the last 25 years (Kantin and Baumgarten, 1982; Yunes et al., 1994; Matthiensen et al., 1999).

The main toxic cyanobacteria reported to bloom in the estuarine region of the Patos Lagoon are species of a

*Corresponding author. Fax: +55 53 32336737.
E-mail address: matthiensen@lycos.com (A. Matthiensen).

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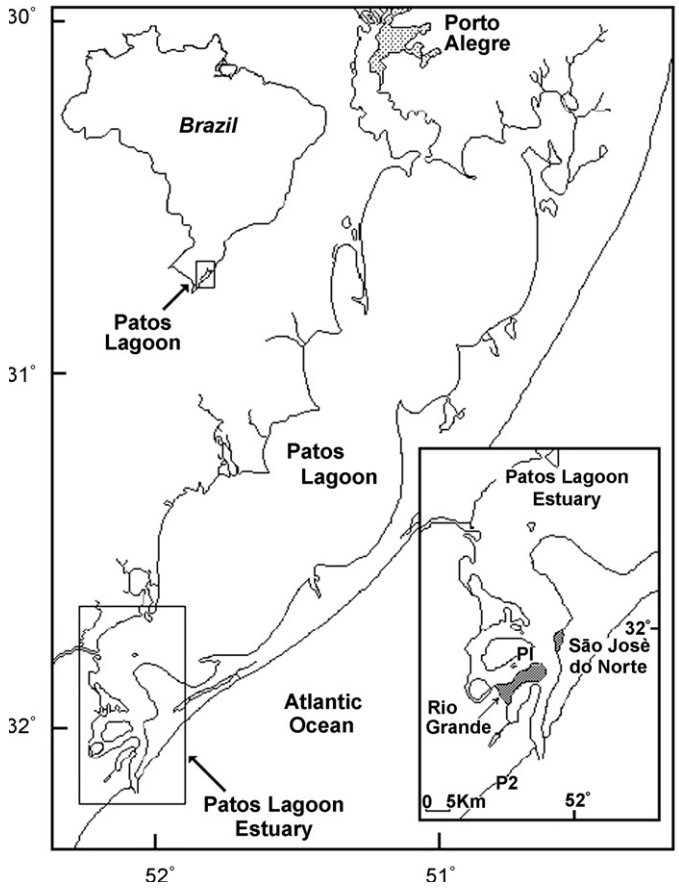

Fig. 1. Maps showing the location of Patos Lagoon and its estuarine system with both water sampling points P1 and P2.

microcystin-producer genus *Microcystis*. Microcystins (MCs) are cyclic heptapeptides containing a characteristic β-amino acid residue, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid (Adda) (Krishnamurthy et al., 1989). They are potent hepatotoxins and tumour promoters (MacKintosh et al., 1990; Falconer, 1991; Carmichael, 1994) and recently it was classified by the International Agency for Research on Cancer as "possibly carcinogenic to humans (group 2B)" (Grosse et al., 2006). Nowadays there are approximately 70 different structural variants known for MCs. The main MC variant occurring at the Patos Lagoon system is the [D-Leu^1]MC–LR (Matthiensen et al., 2000a). The hepatotoxicity of this variant have already been reported for animal and enzymatic bioassays showing that it is one of the most toxic MC variant known (Matthiensen, 2000).

Occurrences of toxic cyanobacterial blooms in several countries in artificial and natural water bodies destined to human consumption (Kuiper-Goodman et al., 1999) resulted in several reports and initial experiments on biodegradation of cyanotoxins (Rapala et al., 1994; Jones and Orr, 1994; Jones et al., 1994; Lam et al., 1995). It is now known that hepatotoxins may suffer biological degradation by aquatic bacteria due to enzymatic pathways reported particularly for some strains of bacteria identified as pertaining to the genus *Sphingomonas* (Bourne et al., 1996; Park et al., 2001; Saitou et al., 2003; Amé et al., 2006). A microcystin-degrading gene cluster, *mlr* A, B, C and D, was identified, sequenced and the degradation process was proposed (Bourne et al., 2001; Saito et al., 2003; Imanishi et al., 2005). For the specific variant [D-Leu^1]MC–LR, it has been reported as biodegraded and/or biotransformed by aquatic microbes from the estuarine region of Patos Lagoon without loss of toxicity assessed by PPIA (protein phosphatase inhibition assay) and without loss of recognition by MC-specific ELISA antibodies (Matthiensen et al., 2000b).

The toxic *Microcystis* blooms that usually occur in the estuarine region of the Patos Lagoon are able to tolerate some level of salinity increases when it is carried out of the estuary and therefore remain for some time in the coastal zone of the adjacent beach (Yunes et al., 1996; Matthiensen et al., 1999). In these occasions, direct contact of the cyanobacterial mass with tourists in this recreational area is very likely to occur with possible harmful results (Falconer et al., 1999). This work aimed to assess MC biodegradation and/or biotransformation by aquatic bacteria from the Patos Lagoon estuary and the adjacent coastal water samples and the isolation and identification of biodegrading responsible bacteria.

2. Material and methods

2.1. Cyanobacteria culture conditions and cell extract preparation

Microcystis strain RST9501 (UPC Culture Collection, FURG, Brazil) was grown in BG-11 medium with nitrate (Stanier et al., 1971) in batch culture at 20–25 °C for toxic cell extract production. Cultures were sparged with air and light was supplied by white fluorescent tubes giving an irradiance incidence on the surface of the vessels of 20 mmol photon m^{-2} s^{-1}. Cells were harvested by centrifugation at 10,000*g* from the stationary phase batch culture for 20 min. Cell pellets were frozen, lyophilised and stored at −20 °C. Extraction and semi-purification of [D-Leu^1]MC–LR were carried out according to Lawton et al. (1994) from lyophilised *Microcystis* RST9501.

2.2. Environmental water sampling

Volumes of 1 L of surface water were collected from the estuarine region of the Patos Lagoon and the marine adjacent coast (P1 and P2, Fig. 1) in mid-July 2004. Water samples were kept in plastic bottles until arriving at the laboratory. Water temperatures were measured *in situ* with a mercury thermometer. Salinity and pH were measured as soon as the samples arrived at the laboratory with a conductivimeter (DM31, Digimed) and pHmeter (Marte, Digimed), respectively. Salinity is expressed in the Particular Salinity Scale (PSS).

2.3. Biodegradation experimental designs

We designed four different experiments. Each experiment was performed in duplicate without shaking in 50 mL amber flasks containing 30 mL of environmental sample (from P1 or P2) plus 30 μg of toxic cell extract or 30 μg of commercially acquired purified MC–LR (Sigma®) to a final concentration of approximately 1 μg mL^{-1} to all flasks. Sub-samples of 0.5 mL were taken from the flasks every 3 or 4 days during approximately 1 month using a micropipette in a laminar flow cabinet