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Highlighted article

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

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Received 14 June 2006; received in revised form 9 March 2007; accepted 24 March 2007

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

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doi:10.1016/j.ecoenv.2007.03.013

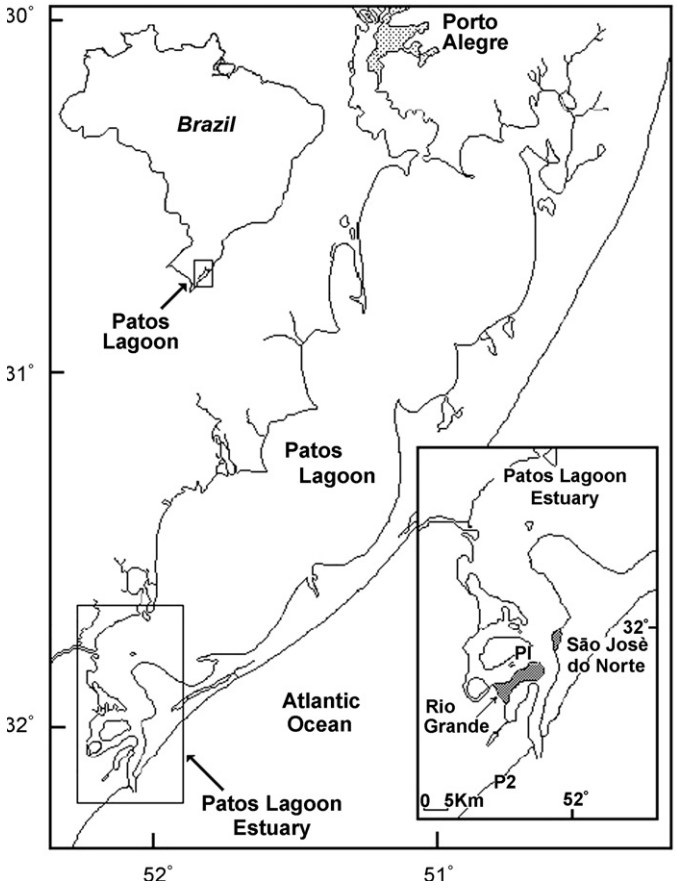

Fig. 1. Maps showing the location of Patos Lagoon and its estuarine system with both water sampling points P1 and P2.

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

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in the presence of a Bunsen burner to keep sterile conditions and the sub-samples were immediately frozen at −20 °C. Sterile (autoclaved) controls were used to quantify the possible abiotic losses due to physical and chemical degradation and especially to evaporation during sub-sampling procedures. All results of biodegradation were obtained in reference to sterile controls.

2.4. Chromatographic analysis

The sub-samples were thawed to room temperature and centrifuged (10,000*g*, 10 min). Volumes of 20 μL were injected into a SCL-10A_{vp} Shimadzu™ HPLC system comprising a LC-10AD_{vp} quaternary pump, UV-SPD 10A_{vp} detector and CTO-10AS_{vp} column oven, controlled by the CLASS-VP 6.21 SP5 software. The analyses were performed with a C_{18} column (Luna® 250 × 4.6 mm, 5 μm particle size) kept at 40 °C. The mobile phase was Milli-Q® water/acetonitrile, both containing 0.05% (v/v) TFA with a linear gradient from 65:35 to 40:60 over 15 min. Chromatogram results were monitored at 238 nm of wavelength and calculated against a standard curve with MC–LR (Sigma®). The variant [D-Leu^1]MC–LR was quantified as MC–LR equivalents.

2.5. Biodegradation rate and exponential decay rate calculation

The average biodegradation rate was calculated dividing the concentration of MC as initially spiked into the samples (either as commercially acquired purified MC–LR or from cell extracted semi-purified [D-Leu^1]MC–LR) by the number of the days until MC was no longer detected by HPLC analysis. The exponential decay rate constants were calculated from a simple first-order rate law.

2.6. ELISA analysis

The enzyme-linked immunosorbent assay (ELISA) was performed according to the kit instructions (Quantiplate™ Kit for Microcystins, Envirologix®, USA). Sub-sample duplicates collected on days 1st and 43rd were diluted with Milli-Q® water to match the sensitivity of the MC–LR standard curve (approximately 4000 ×). The sub-samples were read at 450 nm of wavelength using a tuneable microcavity analyser (Quick ELISA™, Drake®, Brazil).

2.7. Bacterial isolation by enrichment culture technique

Sub-samples from the 43rd day of the experiment was collected by micropipette in a laminar flow cabinet under sterile conditions and inoculated at 26–28 °C on solid Minimal Salt Medium (MSM) (Morsen and Rehm, 1987) plus commercially acquired purified MC–LR at final concentration of 20 μg L^{-1}. The media containing MC–LR as the sole carbon source were used to confirm the biodegradation capability of the isolated strain. The strain that presented growth in MC–LR medium was then re-inoculated in MSM plus D-glucose at final concentration of 8.0 g L^{-1} for maintenance due to the expensiveness of the MC–LR medium.

2.8. DNA purification and PCR reaction

The DNA of the Gram-negative bacterium isolated was extracted as described in Aljanabi and Martinez (1997) with some modifications. A few isolated colonies (up to five, size dependent) were added to 200 μL of extraction buffer (0.4 M NaCl, 10 mM Tris–HCl pH 8.0 and 2 mM EDTA) containing 25 μL lysozyme (10 mg mL^{-1}) and incubated at 37 °C for 1–2 h following an overnight incubation at 62 °C. Protein precipitation was achieved adding 150 μL of saturated NaCl (6 M) and agitating for 30 s. The precipitate was centrifuged (10,000 *g*, 30 min at 4 °C) and 250 μL from the supernatant was transferred to a new 0.5 mL microcentrifuge tube. DNA was precipitated by adding an equal volume of isopropanol and incubating at −20 °C for at least 1 h. The new precipitate was centrifuged (10,000 *g*, 30 min at 4 °C), supernatant was disposed and the pellet was washed once with 500 μL of aqueous ethanol 70% (v/v) and re-centrifuged (10,000 *g*, 10 min at 4 °C). The supernatant was disposed and the pellet was dried for at least 30 min at room temperature. The pellet was resuspended in 50 μL TE (10 mM Tris, 1 mM EDTA, pH 8.0) containing 20 μg mL^{-1} of RNAase and stored at 4 °C.

A partial sequence of 16S rDNA gene was amplified using the primers 1541R 5′AAGGAGGTGATCCAGCC3′ and Epsilon adaptation of 7f 5′GAGASTTTGATCMTGGCTCAG3′ (Embley, 1991). Each PCR reaction had a final volume of 50 μL and contained 5 μL of target DNA, 1 μL of dNTP (10 mM), 1 μL of each primer (10 μM), 1 unit of *Tth* (Biotools®) and 1/10 of reaction buffer containing 2 mM $MgCl_2$. The PCR reaction was performed using the following profile: one hotstart, 5 min at 94 °C, 40 amplification cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 20 s and extension at 72 °C for 40 s. A final extension time of 3 min at 72 °C was added. Results were observed by agarose gel electrophoresis of the PCR product.

2.9. Sequencing and sequence alignment

PCR products were treated with ExoSap (Werle et al., 1994) or gel purified using GFX PCR DNA purification kit (GE-Healthcare®). The same primers used for amplification were used for sequencing. Sequences of PCR products were determined using the DYEnamic ET terminators sequencing kit (GE-Healthcare®) following the protocol supplied by the manufacturer. Sequencing reactions were then analysed in a MegaBACE 500 automatic sequencer (GE-Healthcare®). Each PCR product was sequenced four times in both directions using the same primers. The quality of DNA sequences was checked using BioEdit package (Hall, 2005). Assembled sequences were aligned using CLUSTALX (Thompson et al., 1997) with default gap penalties. Identification of the sequence was performed using BLAST with default settings at the NCBI database and at the Ribosomal Database Project II (RDPII) (Cole et al., 2005).

3. Results

The water temperature and pH values from both sampling sites differed little (14 °C and pH 7.76 for P1 and 15 °C and pH 7.87 for P2). Salinity values about 20 were observed in winter at the coastal zone and reflected the period of high water flow from the lagoon to the sea. Salinity was the environmental variable measured which presented the most different results when comparing both sampling sites (11.2 and 22.1 for P1 and P2, respectively). Despite the difference, similar behaviour was observed for the experiments from both places. Sudden decreases in MC concentrations to both variants were observed in the experiments incubated with water samples from the estuarine (P1) and coastal (P2) regions (Fig. 2). The results are presented as percentage of decreasing MC concentrations to equalise the distinct initial concentrations spiked to the samples. The observed concentration decreases were considered as biological degradation of MCs since the control experiments remained constant during the experimental period. Commercially acquired MC–LR presented an early start on the degradation process in both environmental water samples (4–8 days) while cell extracts showed only degradation after the 15th day. The reducing peak areas to the variant [D-Leu^1]MC–LR is shown in the HPLC chromatograms of Fig. 3. The same chromatograms (Figs. 3B and C) also show the appearance of new peaks at approximately 4.0, 6.5 and 8.5 min, probably as

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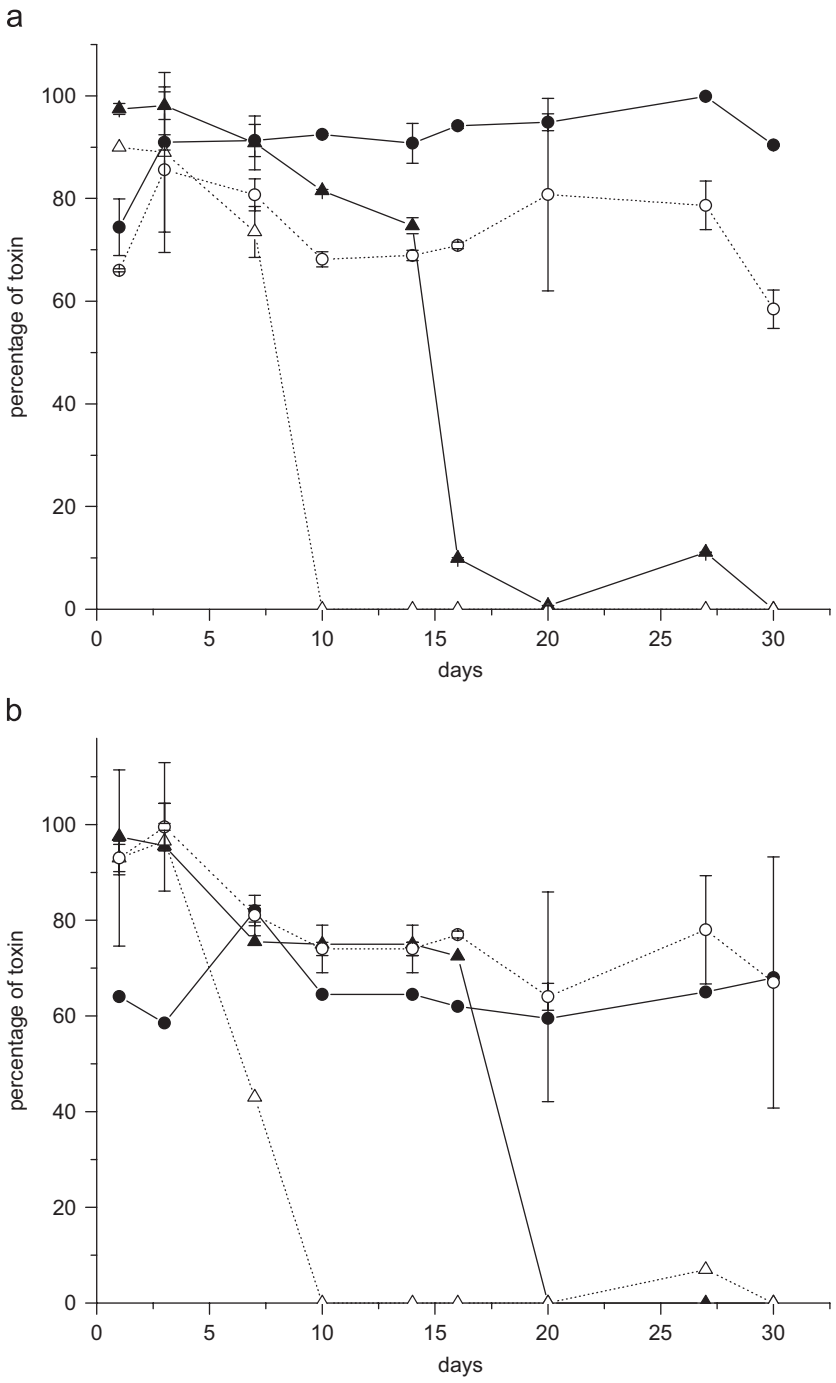

Fig. 2. Percentage of microcystins in the biodegradation experiment series. Solid figures and lines are cell extracted [D-Leu^1]MC–LR; open figures and dotted lines are commercially acquired MC–LR; triangles are experimental and circles are control samples. (a) Water samples from P1 and (b) from P2. All points are means of two observations and vertical bars indicate standard deviation.

intermediate and new compounds resulted from the biological degradation of the [D-Leu^1]MC–LR molecules.

The highest average biodegradation rate was about 0.05 μg MC–LR equivalents $mL^{-1}\,day^{-1}$ observed in the experiment with cell extracts of *Microcystis* RST 9501 in the estuarine water sample (P1). At the same experiment, the first order exponential decay rate has presented a value of about $0.063\,day^{-1}$ (Table 1). The experiment with cell extracts added to marine samples presented lower biodegradation rate values (average 0.0008 μg MC–LR equivalents $mL^{-1}\,day^{-1}$), which would reflect the lower initial MC concentration in relation to the estuarine one (about 40 times lower). However, the exponential decay rate constant over time did not sustain such big difference.

The experiments with commercially acquired purified MC–LR (spiked at similar initial concentrations for both estuarine and marine water samples—about 0.75 μg MC–LR mL^{-1}) showed the same average

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biodegradation rate (0.08 μg MC–LR mL^{-1} day^{-1} for both water samples) and similar exponential decay rate constants (0.174 and 0.201 day^{-1}; Table 1).

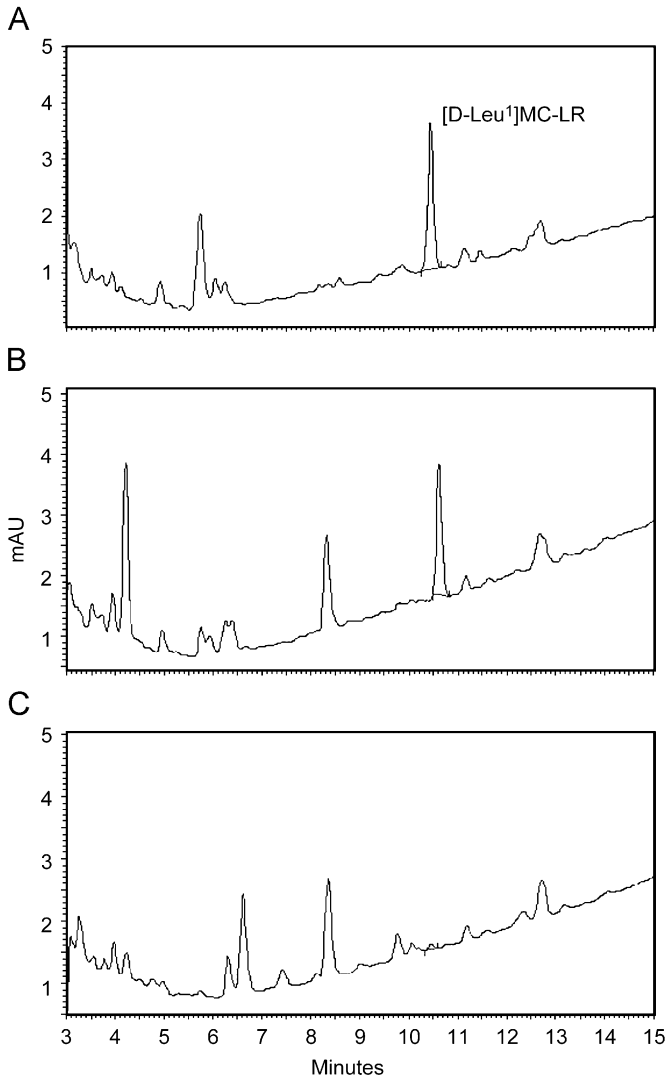

Fig. 3. Chromatograms showing the decreasing peak of [D-Leu^1]MC–LR from estuarine water of Patos Lagoon. (A) 1st day; (B) 16th day; and (C) 30th day of experiment.

Table 2 shows the percentage of MC concentrations measured by ELISA test kit at the first day and after 43 days from the beginning of the experiment. The highest value observed for each sample on the ELISA analysis at the first day was considered 100% of MC spiked in the water sample (as commercially acquired MC–LR for the experiments with purified toxin and as [D-Leu^1]MC–LR for the experiments with cell extracts). After 43 days it was observed that the ELISA antibodies cross-reacted with MC molecules only up to 8.6% from the initial concentration measured (water sample from P2, cell extract experiment). According to the data from Table 2 more than 90% of the total MC analysed from all experiments was absent, and in some cases up to 99% (MC–LR from P1 water samples). This confirms that the MC molecular structures were no longer recognised by the MC specific antibodies from the ELISA test kit.

After confirmation of the MC biodegradation from all the experiment series tested, sub-samples were inoculated from all the resulting experimental material to MSM plus MC–LR agar media. After several successive re-inoculations to guarantee that no bacteria strain was growing at expense of organic carbon traces resulting from the medium transferences, a yellow Gram-negative rod-shaped cell bacteria strain was observed to grow.

A bacterium strain was isolated from P1 water sample growing in the MSM plus MC–LR agar medium. This strain was identified by partial sequencing of the 16S

Table 2
ELISA results expressed as percentage of quantified toxin[a]

Water sampling points	Experimental series	1st day (%)	43rd day (%)
P1	MC–LR	95.9±5.82	0.6±0.78
	[D-Leu^1]MC–LR	95.4±6.48	5.3±3.90
P2	MC–LR	90.4 ±13.61	2.0±2.83
	[D-Leu^1]MC–LR	93.1±9.77	8.6±0.06

100% was the highest value quantified for each experiment on the 1st day of analysis.

[a]Data presented are means of duplicate measurements±SD.

Table 1
Initial concentrations, biodegradation rates and exponential decay rate constants of microcystin variants measured during the biodegradation experimental series[a]

Water sampling points	Experimental series	Initial concentration[b]	Average biodegradation rate[c]	Exponential decay rate constant[d]
P1	MC–LR	0.77±0.00	0.08±0.00	0.174±0.013
	[D-Leu^1] MC–LR	0.89±0.009	0.05±0.0005	0.063±0.008
P2	MC–LR	0.75±0.00	0.08±0.00	0.201±0.004
	[D-Leu^1] MC–LR	0.02±0.001	0.0008±0.00003	0.053±0.0001

[a]Data presented are means of duplicate measurements±SD. MC–LR, commercially acquired purified MC–LR; [D-Leu^1]MC–LR, cell extract semi-purified [D-Leu^1]MC–LR.

[b]μg MC–LR ml^{-1} (or MC–LR equivalents, in case of the variant [D-Leu^1]MC–LR).

[c]μg MC–LR ml^{-1} day^{-1} (or MC–LR equivalents, in case of the variant [D-Leu^1]MC–LR).

[d]Calculated from first-order exponential decay rate over time (day^{-1}).

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rDNA gene as a bacterium belonging to the β-proteobacteria group from the genus *Burkholderia*, with similarity of 96% using the RDPII and NCBI database. The partial sequence obtained was deposited in the GenBank under accession number DQ459360.

4. Discussion

Microbial populations from the Patos Lagoon estuary system have managed to breakdown MC molecules. When the results of MC degradation are compared to the control samples it can be assumed that there was no abiotic degradation due to physical and/or chemical factors. It was observed complete degradation of MC molecules in all samples tested and analysed by HPLC, as it was also suggested by the ELISA results.

The time period in which total biodegradation was observed (10–15 days) was similar to the period reported previously (Matthiensen et al., 2000b) for the Patos Lagoon estuarine waters, but in that occasion the remaining degraded molecules retained toxicity assessed by PPIA analysis. Also, average biodegradation rates of about 0.03–0.06 μg MC–LR mL^{-1} day^{-1} were observed for 5 different places inside the estuary of the Patos Lagoon varying salinity from 3.7 to 14.8, which is in accordance to our present observations (Matthiensen et al., 2000b). In one sample we have observed a much lower rate (semi-purified [D-Leu[1]]MC–LR extract added to P2 samples) but it was attributed to the low initial MC concentration added to the vessels, since the exponential decay rate constant was fairly close to the same rate constant in P1 samples (Table 1). Together with the previous results (Matthiensen et al., 2000b) it can be suggested that similar biodegradation rates were observed when initial MC concentrations were similar, independently of the estuarine region where sampling took place. We may conclude that, in terms of biodegradation from the aquatic bacteriological community specifically on the MC-degrading species, there was little spatial variability when confronted against salinity, which was the main environmental variable measured for water mass differentiation.

The initial concentration of toxins in the beginning of the experiments may become an important factor regarding of how fast they can be degraded. Once a bacterial population is established and manages to breakdown a formerly recalcitrant compound, they will do it as fast as possible, probably in response to the lack of more convenient food sources. It seems obvious that purified MCs degrade faster than the semi-purified toxic cell extracts (not taking into account the structural differences in the MC variants tested). The not-completely purified material offers a bigger diversity of compounds as possible food source to be degraded first. MCs are believed to be very stable molecules, so it is natural that the presence of other molecules would confers a delay in the degradation of MCs.

The ELISA results confirmed the MC degradation observed by the decreasing of the peak areas in the HPLC chromatograms. The non-recognition and cross-reaction with the MC antibodies in the ELISA indicate that the former compounds are no longer MCs or related compounds. This result is different from previously described results by Matthiensen et al. (2000b) where after 33 days the ELISA results still confirmed the biotransformed products as MC molecules, although decreasing MC concentrations where detected by HPLC. If the newly degraded molecules still remain toxic and which organisational level of toxicity it would affect is unknown. Due to the small volume resulting from the original materials after the biodegradation and isolation experiment series, it was impossible to perform further toxicity tests.

The isolation of a bacterium from the genus *Burkholderia* from an MC containing sample is not surprising since species from this genus have been described for different contaminated environments. Although we have not determined the complete 16S rDNA sequence of the biodegrading organism, we can quite confidently place it in the genus *Burkholderia*. Since the *Burkholderia* genus contains over 34 described species (Payne et al., 2005; Belova et al., 2006) that occupy very diverse ecological niches (contaminated soils, aquatic environments and the respiratory tract of humans) it is probably necessary to use a more specific marker to determine if our organism is a new species within this genus.

The main known *Burkholderia* species is *B. cepacia*, a nonfermentative aerobic Gram-negative bacillus, formerly classified as *Pseudomonas*. However, *B. cepacia* is not a single microorganism, but rather a complex of related species or genomovars referred to as the *B. cepacia* complex (Bcc) (Coenye and Vandamme, 2003; Payne et al., 2005). This group comprises at least nine species sharing a high degree of 16S rDNA (98–100%) and *recA* (94–95%) sequence similarity, and moderate levels of DNA-DNA hybridisation (30–50%) (Coenye and Vandamme, 2003). These bacteria possess multiple chromosomes and unusually large genomes, carrying great genetic potential for adaptation to many different environments (Lessie et al., 1996; Valvano et al., 2005). Although it was impossible for us to determine the *Burkholderia* species as a certainty, the isolated bacterium is most likely a member of the *B. cepacia* complex.

Bcc seems to have the ability to metabolise virtually anything available to it (Johnson and Olsen, 1997; Balashova et al., 1999; Inguva and Shreve, 1999; Bhushan et al., 2000; Leahy et al., 2003; Chaillan et al., 2004; Olaniran et al., 2004; Tillmann et al., 2005), and Bcc isolates have already been exploited for several purposes such as biological control of plant pathogens, bioremediation of recalcitrant molecules and plant growth promotion (Coenye and Vandamme, 2003). Some *Burkholderia* strains such as JS150 (ex *Pseudomonas* sp. JS150) are known to have multiple oxygenase pathways for the dissimilation of aromatic compounds, which may further expand the range

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of substrates that can be co-oxidised (Haigler et al., 1992; Johnson and Olsen, 1997). But while several *Burkholderia* strains with potential applications in bioremediation are well-studied regarding their potential in the biotechnological approach, they also are taxonomically poorly characterised, even in clinical microbiology (Van Pelt et al., 1999), and there are much less systematic studies with regard to the distribution of the Bcc species in environmental samples (Bevivino et al., 2002; Miller et al., 2002; Coenye and Vandamme, 2003). However, there exists a growing interest in the development of reliable methods for rapid and efficient identification of bacterial species from environmental samples.

In terms of bioremediation tools, we believe that the biodegradation rates reported here do not reflect the highest values that can be obtained. Biodegradation rates have been shown the tendency to be higher as higher the initial MC concentration. The rate and extent of degradation under determined circumstances is affected by the concentration and availability of all of the mixture components as well as their oxidation products (Leahy et al., 2003). Further experiments properly focused on this subject and biodegradation comparisons with *B. cepacia* strains from different reference cultures will surely bring new answers to it.

The exceptional metabolic versatility of Bcc bacteria can be used as potential tools for bioremediation of contaminated sites, and it presents as an interesting alternative for the recalcitrant MC molecules. The frequent reports of toxic cyanobacterial bloom occurrences worldwide are alarming, and it is growing as fast as grows the eutrophic state of some natural and artificial water bodies and reservoirs. New tools for the bioremediation of affected sites are at sight, and the use of environmental compound-degrading bacteria can be one of them.

5. Conclusion

We confirmed biodegradation of two microcystin variants, MC–LR and [D-Leu[1]]MC–LR, by the aquatic bacterial community of estuarine and coastal waters of the Patos Lagoon estuarine region. We also isolated a bacterial strain able to grow on a media enriched with purified MC–LR as the sole carbon source. This bacterial strain was identified as pertaining to the group of the β-proteobacteria genus *Burkholderia*. As far as we know, this is the first report linking the genus *Burkholderia* to the degradation of microcystins.

Acknowledgments

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support to this work.

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Please cite this article as: Lemes, G.A.F., et al., Biodegradation of microcystins by aquatic *Burkholderia* sp. from a South Brazilian coastal lagoon, Ecotoxicol. Environm. Safety (2007), doi:10.1016/j.ecoenv.2007.03.013

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