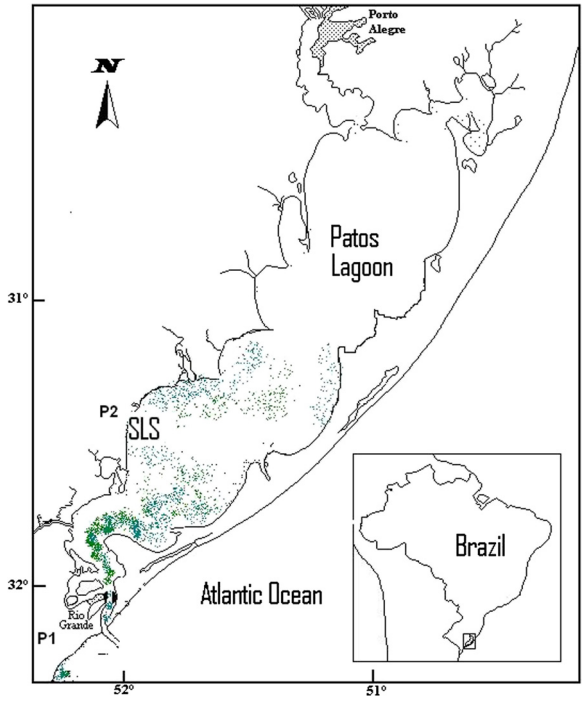

Figure 3 Map of Patos Lagoon estuarine area with collection spots P1 and P2 (Brazil).

During the experiment, samples were collected for analysis every three days, on average; all handling was done in a microbiological chamber with a Bunsen burner. Total sampling time was 30 days. Medium samples were labeled and stored in a freezer at −20°C for further analysis by ELISA immunoassay.

Selection and isolation of the strain to perform the biodegradation assay

At the end of the experiment with sediment, when the concentration of the toxin [D-Leu^1] microcystin-LR reached "zero", a sample of this bacterial strain was removed from the flasks and inoculated in MSM on a Petri dish with the addition of 8 g.L^{-1} glucose anhydrous and 15 g.L^{-1} agar. The strain was then denominated DMXS. In order to guarantee the purity of the isolated, the strain was inoculated in Petri plates containing growth media with pure D-Leu microcystin-LR. A pure isolate was considered a colony (single form) which was repeatedly replicated successfully.

Table 1 Bacteria isolated from water samples collected at two spots in Patos Lagoon: P1, in the Patos Lagoon estuary (near the Oceanographic Museum) and P2, on São Lourenço Beach (Rio Grande do Sul state, Brazil)

Bacterial strain	Spot	Color	Source
BM12	P1	Yellow	Water
EE1	P1	White	Water
$EBDE_1Br$	P2	White	Water
LPML	P1	Orange	Water
NOT13	P1	Yellow	Water
CEV	P1	Red	Water
BB0412	P2	Yellow	Water
FB0607	P1	Red	Water
DMXS	P1	Light brown	Sediment

Evaluation of toxin [D-Leu^1] microcystin-LR concentration by Elisa Immunoassay

Assessment of toxin levels was carried out by ELISA immunoassay with specific antibodies for microcystins. This test has high sensitivity and detects toxins in concentrations as low as 0.05 up to 2.5 μg.L^{-1}. The ELISA kit (Abraxis, USA) was used in accordance with the methodology recommended by the manufacturer.

Bacterial count

Bacterial count was performed through epifluorescence microscopy in a Zeiss Axioplan microscope (Germany) and using the software UTHSCSA Image Tool (Version 3.0) (http://compdent.uthscsa.edu/dig/download.html). Samples were filtered through polycarbonate membranes (Nuclepore, Whatman, UK; pore 0.2 μm) and colored with Irgalan black. Samples were then dyed with acridine orange in accordance with the methodology proposed by Hobbie *et al.* [26] to count bacteria in samples fixed with Lugol's solution [27,28]. This fluorochrome emits green or orangish red fluorescence when it binds to bacterial DNA or RNA, respectively. We took advantage of the image by dividing the screen in several equal squares. For each screen containing an image divided in more than a 100 squares, we choose randomly a minimum of 30 squares to be counted.

Molecular identification

DNA was extracted from 1-mL cell cultures by the Wizard Genomic DNA Purification kit (Promega, USA) in accordance with the supplier's instructions. Extraction products were visualized on 1% agarose gel with GelRed (Biotium, USA). Primers of 16S segment (forward 5′-CCTACGGGAGGCAGCAG-3′ and reverse 5′-GAC TACCAGGGTATCTAATC-3′) were designed as previously described [29]. DNA sample was amplified through polymerase chain reaction (PCR), which was performed in accordance with Ritchie *et al.* [29], except for the primer annealing temperature, which was optimized for 58°C. PCR products (approximately 400-bp long) were analyzed on GelRed-stained 1% agarose gel, with Low DNA Mass Ladder (Invitrogen, Thermo Fisher Scientific, USA) as the molecular weight marker, and then purified by enzymes exonuclease I