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An effective pathway for the removal of microcystin LR via anoxic biodegradation in lake sediments

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ABSTRACT

Aerobic biodegradation has been considered to be the main attenuation mechanism for microcystins, but the role of anoxic biodegradation remains unclear. We investigated the potential for anoxic biodegradation of microcystin and the effects of environmental factors on the process through a series of well-controlled microcosm experiments using lake sediments as inocula. Microcystin LR could be degraded anoxically from 5 mg L^{-1} to below the detection limit at 25 °C within 2 days after a lag phase of 2 days. The rate was highly dependent on temperature, with a favorable temperature range of 20–30 °C. The addition of glucose or low levels of NH_4-N had no effect on the anoxic biodegradation of microcystin, whereas the addition of NO_3-N significantly inhibited the biodegradation at all experimental concentrations, and the inhibition increased with increasing amount of NO_3-N-amended. Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid), a previously reported nontoxic product of aerobic degradation of microcystin, was identified as the anoxic biodegradation product. This is the first report of Adda as a degradation product of microcystin under anoxic conditions. No other product containing Adda residue was detected during the anoxic degradation of microcystin. These results strongly indicated that anoxic biodegradation is an effective removal pathway of microcystin in lake sediments, and represents a significant bioremediation potential.

1. Introduction

Microcystins (MCs) are a group of cyclic heptapeptide hepatotoxins mainly produced by freshwater cyanobacteria. They are responsible for liver failure in wild animals, livestock and aquatic organisms (Carmichael, 2001). MCs have also been attributed to human illnesses (and even death) due to exposure to hepatotoxin-contaminated water (Azevedo et al., 2002). More than 70 variants of MCs have been isolated and identified, among which microcystin LR (MCLR) is one of the most commonly occurring variants. Because MCs in water bodies may pose a potential health risk to humans who come into contact with water (Ueno et al., 1996), scientists and water authorities must understand the natural degradation and elimination pathways of MCs with the aim of reducing the risks associated with these pollutants. Of the five pathways proposed to account for natural reductions in MC levels (dilution, adsorption, thermal decomposition aided by pH, photolysis, and biological degradation) (Tsuji et al., 2001), biodegradation appears to be the primary potential elimination pathway for MCs in freshwater (Holst et al., 2003; Chen et al., 2008).

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Aerobic biodegradation of MC as a major attenuation mechanism for MC in the environment has been widely studied. This process was reported to occur in diverse ecosystems such as sewage effluent (Lam et al., 1995), sand filters (Ho et al., 2007b), reservoirs (Cousins et al., 1996; Valeria et al., 2006), rivers (Bourne et al., 2006) and lakes (Hyenstrand et al., 2003; Chen et al., 2008). The degradation rate is affected by several environmental factors such as temperature (Park et al., 2001), nutrient conditions (Surono et al., 2008), and pre-exposure to MC (Rapala et al., 1994; Ho et al., 2007b). The half-life of MC usually ranges from a few hours to 3 weeks (Lam et al., 1995; Holst et al., 2003; Ho et al., 2007b). One pathway for MCLR biodegradation has been elucidated, which consists of sequential enzymatic hydrolyses of the peptide bonds of Arg–Adda, Ala–Leu, and then Adda–Glu to produce linear MCLR, a tetrapeptide, and Adda (Harada et al., 2004; Imanishi et al., 2005). The fact that the three products are nontoxic compared with parent MCLR (Bourne et al., 1996; Harada et al., 2004) suggests that aerobic biodegradation is a safe and practical process for removing MC from water (Ho et al., 2007a).

Unlike aerobic degradation, anoxic and anaerobic degradation has been regarded to be a negligible attenuation mechanism for MC in the environment. The study of Holst et al. (2003) showed that anoxic microbial degradation of MC is substantial under nitrate-reducing conditions. This finding suggests that anoxic degradation may have a more important role in the fate of MC than previously thought, but until now no further study has been reported and very little is known about this process. It remains unclear if the potential for MC biodegradation under anoxic conditions is ubiquitous in the environment, and how environmental factors affect anoxic biodegradation. Which products are produced in this process and whether these degradation products are toxic is not known.

In the present study, the degradation characteristics of MCLR were investigated under anoxic conditions using lake sediments as the inocula. The effects of environmental factors such as oxygen content, temperature, and amended nutrients on MC degradation were then studied. Finally, the degradation product was isolated, purified and identified by electrospray ionization tandem mass spectrometry (ESI–MS/MS). Results from this study will provide insights into the role of anoxic degradation on the fate of MC, and expand understanding of the natural attenuation mechanism for MC in the environment.

2. Materials and methods

2.1. Standards and reagents

The MCLR standard for analysis was purchased from Sigma–Aldrich (St. Louis, MO, USA). MCLR used for biodegradation experiments was isolated and purified from a laboratory mass culture of *Microcystis aeruginosa* PCC 7806. The isolation procedure involved extraction of cultured cyanobacterial cells with 75% methanol, followed by preparative reversed-phase flash chromatography and semi-prep liquid chromatography (LC) (Waters 600, Basking Ridge, NJ, USA). The purity of MCLR obtained was $\geq$ 95% as determined by high-performance liquid chromatography–diode array detection (HPLC–DAD). The purified MCLR was concentrated and stored at −20 °C. ODS Sep-pak cartridges were obtained from Waters (Milford, MA, USA). HPLC-grade methanol (Tedia Company, Incorporated, Fairfield, OH, USA) was used as the HPLC mobile phase and extraction solvent. All other chemicals were of analytical grade.

2.2. Sediment sample

Samples of surface sediment were collected from Fubao Bay in the northern region of Lake Dianchi (Kunming, Yunnan, China), where heavy cyanobacterial blooms have frequently occurred during the past 20 years, by a stainless steel grab sampler in December 2006. The sediment was air-dried, crushed, and passed through a 100-mesh sieve. It was stored in plastic bags at 4 °C before use. The contents of nitrate (NO_3-N) and total organic carbon (TOC) in air-dried sediment were 18.6 $\mu g\, g^{-1}$ and 170.6 $\mu g\, g^{-1}$, respectively.

2.3. MC biodegradation

Anoxic MC biodegradation experiments were carried out in a series of 25-mL brown glass bottles sealed with rubber stoppers. In each bottle, sediment (0.4 g) and sterilized distilled water (20 mL) were added and mixed, followed by the addition of MCLR to produce a final concentration of 5 $mg\, L^{-1}$. Anoxic conditions were obtained by flushing high-purity nitrogen through the water–sediment mixtures with a needle until the oxygen meter (Oxi 315i, WTW, Weilheim, Germany) indicated that oxygen saturation was $<$ 1%. The bottles were then sealed with stopples and wax. They were incubated under anoxic conditions in the dark at 25 °C ($\pm$ 0.5 °C). For each treatment, 0.5 mL of well-mixed sample was collected from each bottle at different time intervals with a needle and syringe. After centrifugation at 10,000 $\times$ *g* for 15 min at room temperature, supernatants were transferred to HPLC autosampler vials for determination of the concentration of MCLR and its degradation products. Before and during sampling, high-purity nitrogen was sparged into bottles to maintain anoxic conditions. Autoclaved sediments and water were the controls for non-biological removal of MC. Experiments were carried out in duplicate.

Different treatments were as follows: aerobic biodegradation of MC was done by incubating bottles sealed with a cotton plug on a rotating plankton wheel (oxygen saturation, 96%); the effect of temperature on anoxic MC degradation was studied by incubation at 10, 15, 20, 25 and 30 °C; the influence of amended nutrients on anoxic biodegradation of MC was investigated by addition of $C_6H_6O_6$ (0–1000 $mg\, L^{-1}$), NH_4Cl (0–1000 $mg\, L^{-1}$), or $NaNO_3$ (0–1000 $mg\, L^{-1}$). These experiments were carried out with the same protocol as described in the previous anoxic experiments, and experimental parameters were identical to those previously described except those specified.

2.4. Isolation and purification of degradation product

After MCLR concentration in the anoxic biodegradation experiment decreased to an undetectable level, the