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Seasonal dynamics of microcystin-degrading bacteria and toxic cyanobacterial blooms: Interaction and influence of abiotic factors

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ABSTRACT

Massive proliferations of cyanobacteria coexist and have different interactions with other microorganisms, including microcystin (MC)-degrading bacteria. Despite their relevance in the environment for the removal of MCs, this bacterial community has been scarcely studied. The influence of physicochemical factors and the seasonal dynamics of toxic cyanobacteria on the relative abundance and seasonal dynamics of the MC-degrading bacterial community with *mlr* genes (*mlr*$^+$) were investigated during a two-year study at a water reservoir in central Spain. The capacity of the total bacterial community on the degradation of MCs during the whole period of study was also evaluated. The results showed that the relative abundance of *mlr*$^+$ bacteria started to increase after the increase in the relative abundance of toxic cyanobacteria and MC concentrations in the water, indicating a related seasonal dynamic and an important interaction between the two communities. The correspondence of several peaks of *mlr*$^+$ bacteria with decreases in the relative abundance of toxic cyanobacteria and vice versa may also suggest a possible antagonistic relationship that deserves an in-depth study. The lack of a significant relationship between the physicochemical factors and the temporal shifts of both MC producers and degraders also supports the notion that the interaction of the two communities is an important driver of their seasonal dynamics in nature. Regarding the capacity of the total bacterial community for the degradation of MCs, this capacity was only observed during the toxic cyanobacterial bloom episodes, highlighting the importance of the pre-exposure to MCs in the reservoir for triggering the MC biodegradation process.

1. Introduction

The occurrence of massive cyanobacterial growth due to eutrophication is becoming a prevalent situation worldwide, causing water quality problems for recreational and domestic uses. Moreover, climate change predictions point towards an elongation of the growing season with extended periods of cyanobacterial dominance (Paerl et al., 2011) and the disruption of the ecosystem function by uncoupling trophic relationships (De Senerpont Domis et al., 2013, 2007). The dominance of microcystin (MC)-producing cyanobacteria and the associated development of hepatic diseases, irritant reactions and tumour-promoting activities (Falconer and Yeung, 1992; Ueno et al., 1996) have increased the concern of water authorities and health organizations. The MCs are the most frequent and widespread toxins produced by several freshwater cyanobacterial genera, including *Microcystis*,*Dolichospermum* and *Planktothrix*, (Sivonen and Jones, 1999) and are synthesized by multienzyme complexes encoded by the *mcy* gene cluster (Börner and Dittmann, 2005). The molecule of MC consist of seven amino acids formed in a cyclic structure that is stable in water and resistant to some physicochemical processes (Chen et al., 1998; Harada et al., 1996; Tsuji et al., 1994). Some freshwater bacteria, however, are able to degrade MCs efficiently under environmental conditions (Jones and Orr, 1994; Kormas and Lymperopoulou, 2013). An enzymatic pathway encoded by the *mlr* gene cluster has been found to be responsible for the breakdown of MCs (Bourne et al., 2001). The *mlr* gene cluster has been reported to be constitutive (Alamri, 2010; Bourne et al., 2001; Ishii et al., 2004) although upregulated with the MC concentrations, thus showing higher transcriptional responses with increased MC concentrations (Jiang et al., 2011). Recent studies, however, have suggested the existence of alternative-*mlr* MC degradation pathways operating in nature. The absence of *mlr* genes in some isolated MC-degrading bacteria (Lezcano et al., 2016; Manage et al., 2009),

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and the relationships observed between *mlr*-lacking bacteria able to degrade organic complex compounds and the toxic cyanobacterial blooms (Lezcano et al., 2017; Mou et al., 2013), support the hypothesis that there are bacteria lacking *mlr* genes involved in the degradation of MCs in the environment.

The dominance of cyanobacteria within the phytoplankton community, as well as the temporal shifts in the cyanobacterial composition, has been traditionally believed to be driven by bottom-up control factors (abiotic). Light, nutrients and temperature have been reported to influence the growth of cyanobacteria, the production of MCs (Monchamp et al., 2014; Orr and Jones, 1998; Sivonen, 1990; Vézie et al., 2002) and the expression of the *mcy* gene cluster (Kaebernick et al., 2000; Sevilla et al., 2008). However, top-down control factors, such as interactions with other organisms (virus, fungi, bacteria and zooplankton), are receiving special attention to understand the success of the cyanobacterial blooms and the variations in their toxicity (Sønstebø and Rohrlack, 2011; Van Wichelen et al., 2016; Wilken et al., 2014). Although the heterotrophic bacteria associated with toxic cyanobacterial blooms have been reported to have different types of interactions with cyanobacteria, such as parasitic, predatory or mutualistic (Rashidan and Bird, 2001; Van Wichelen et al., 2016), the ecological significance of the specific MC-degrading bacterial community has hardly been studied and, hence, is poorly understood in nature. As far as we know, only one previous study performed by Zhu et al. (2014) studied the seasonal dynamics of the potentially MC-degrading bacteria with *mlr* genes (*mlr*$^+$) in the environment. The study showed an increase in the *mlr*A gene abundance after an increase in the MC concentrations during a toxic cyanobacterial bloom, pointing towards a close interaction between the two communities. The scarce number of studies performed on the seasonal dynamics of *mlr*$^+$ bacteria, as well as the currently unknown influence of the physicochemical factors on their temporal shifts, are hindering the understanding of the relationship between the *mlr*$^+$ bacteria and the toxic cyanobacterial blooms in nature.

Moreover, several studies performed in the laboratory have reported a link between the pre-exposure of the bacterial communities or cultures to MCs and their MC biodegradation rates (Ho et al., 2007; Morón-López et al., 2017).The MC degradation capacity and efficiency of the bacterial communities in the environment before, during and after a naturally occurring toxic cyanobacterial bloom is however still poorly understood and deserve in-depth study.

Therefore, the present study has the following aims: i) to determine the influence of the physicochemical factors and the seasonal dynamics of the MC-producing cyanobacteria on the relative abundance and seasonal dynamics of the *mlr*$^+$ bacterial community and ii) to assess the MC degradation capacity and efficiency of the total bacterial community before, during and after toxic cyanobacterial bloom episodes. To achieve this, the most relevant physicochemical factors (temperature, pH, dissolved oxygen and nutrients) and the relative abundance of both potentially MC-producing (*mcy*E gene as a marker) and MC-degrading (*mlr*A gene as a marker) bacterial communities were monitored over two years in a reservoir in central Spain. To assess the MC degradation capacity of the total bacterial community, *in vitro* MC-degradation assays were performed with the collected water samples.

2. Materials and methods

2.1. Sampling setup

The study was conducted in the San Juan reservoir, a waterbody located in central Spain (Madrid) with a history of persistent toxic cyanobacterial blooms. Water samples were periodically collected from the subsurface in one point located approximately two meters from the shoreline (40° 22′ 44.10″ N and 4° 19′ 40.95″ W) from 20 August 2013 to 28 January 2015. From the start of the cyanobacterial bloom in late summer until after the collapse of the bloom in late autumn, samples were collected fortnightly to monitor thoroughly the MC-producing and MC-degrading bacterial communities. During the cold period and low MC concentrations, samples were collected bimonthly and then changed to monthly during the summer before the onset of the cyanobacterial bloom. For an overview of the seasonal precipitation, temperature and light duration in the sampling point, see Table S1. The measurements of the physical and chemical factors were performed *in situ* using portable individual probes for dissolved oxygen (Crison OXI 45 P, Hach Lange, Spain), pH (Crison pH 25 Hach Lange, Spain) and temperature. Each sample was comprised of 4 L of subsurface water collected with sterilized polyethylene bottles and stored in dark and cold (4 °C) conditions during transport (less than 2 h) to the laboratory. The samples were homogenized, split into four aliquots and treated with different protocols. One litre of water was sequentially filtered through 2-μm (25 mm, Whatman, Maidstone, United Kingdom) and 0.22-μm (25 mm, Millipore, Darmstadt, Germany) pore size polycarbonate filters to collect the larger and smaller fractions of the cyanobacteria and other aquatic bacteria (both those attached to the cyanobacterial mucilage and those free-living), respectively. The filter membranes were stored at −20 °C for further DNA analysis. The second litre of water was filtered through fibreglass filters (approximately 0.7 μm, Millipore) to collect seston and then stored at −20 °C for subsequent MC and chl-*a* quantification. The flow-through was also collected and preserved at −20 °C for the analysis of dissolved MC and nutrient concentrations (Dissolved Organic Carbon (DOC), nitrogen from nitrate ($N\text{-}NO_3^-$), phosphorus from soluble phosphate ($P\text{-}PO_4^{3-}$)). The third litre of water was immediately used to test the *in vitro* MC degradation capacity and the efficiency of the total bacterial community. The fourth litre was used for the identification of cyanobacteria under a microscope (Olympus CX41, Tokyo, Japan) after 24 h of flotation (Cirés and Quesada, 2011).

2.2. Nutrient analysis

The water samples that were filtered through fibreglass filters (approximately 0.7 μm) for the analysis of DOC, $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$ were filtered again through 0.45-μm nylon syringe filters (25 mm, LLG, Meckenheim, Germany) before analysis. The analysis of DOC was performed in a Total Organic Carbon analyser (TOC-V CSH, Shimadzu, Japan) after previous digestion of the samples by burning at 680 °C and subtracting the inorganic carbon fraction. The analysis of nitrate was performed in an 861 Advanced Compact IC system (Metrohm, Switzerland) with an autosampler 838 Advanced Sample Processor (Metrohm, Switzerland). Chromatographic separation was performed using a Metrosep A Supp 7-250 column (Metrohm, Switzerland) thermostated at 45 °C. The mobile phase consisted of 3.6 mM Na_2CO_3 with a flow rate of 0.7 mL min^{-1}. The analysis of soluble phosphate was performed following the ascorbic acid method described in the American Public Health Association – American Water Works Association Standard Methods (AWWA, 1992).

2.3. MC and chl-a extraction from water samples

Sestonic MCs and chl-*a* from fibreglass filters were extracted in duplicate and double-extracted by sonication (P-Selecta Ultrasons, Barcelona, Spain) with 90% aqueous methanol. Chl-*a* was immediately quantified using spectrophotometric measurement at 665 nm (Shimadzu Multispec-1501, Kyoto, Japan). The