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Bacterial communities' response to microcystins exposure and nutrient availability: Linking degradation capacity to community structure

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ABSTRACT

Eutrophication of freshwater bodies followed by cyanobacterial bloom and toxin production is an important issue in freshwater supply in both developed and developing countries. The primary mechanism for microcystins (MCs) (the main class of cyanobacterial toxins) dissipation is microbial degradation. Repeated exposure of freshwater bodies to cyanobacterial toxins MCs may affect indigenous microbial communities and may also enhance biodegradation of MCs, but the factors driving this relationship remain unclear. Six Scottish freshwater bodies with different histories of natural exposure to MCs and ability to degrade MC-LR (the most common microcystin) were chosen as case study. Terminal Restriction Fragment Length Polymorphism (T-RFLP) and Biolog EcoPlate™ were used to study the structure and physiology of the bacterial communities. Previous exposure to MCs significantly contributed to the bacterial communities shape and microbial physiology of the water bodies under study. Other factors that significantly affected the bacterial communities were dissolved organic carbon and concentration of nitrogen compounds as well as temperature. Moreover a significant relationship was found between bacterial communities' structure and MC-LR half-life. These data suggest that exposure to MCs drives changes in structure and physiology of bacterial communities and in turn those communities differentially perform degradation of MC-LR.

1. Introduction

Freshwater is one of the most precious natural resource on the planet. Natural waters have very low concentrations of nitrates and phosphorous. Runoff from farm lands, along with wastewater deriving from urban and industrial activities increase nutrient loads. Eutrophication, a higher nutrient (e.g. nitrates and phosphorus) concentration, and high temperatures stimulates cyanobacterial blooms of inland water bodies (Chorus and Bartram, 1999; Sharpley et al., 2003). The blooms represent an overgrowth of cyanobacteria, which are a diverse group of ancient autotrophs that occur globally. The increased incidence of toxic cyanobacterial blooms represent a hazard for human and animal health (Chorus and Bartram, 1999; Chorus et al., 2000). The toxicity of the cyanobacterial bloom is due to the presence of a wide range of toxins produced by cyanobacteria: microcystins and nodularins (hepatotoxins and carcinogens), saxitoxins and anatoxins (neurotoxin), and cylindrospermopsin (protein synthesis inhibitor) (Edwards and Lawton, 2009). Microcystins (MCs) are the most common cyanotoxins and may be expected wherever blooms of cyanobacteria occur in surface water. Their occurrence is highly likely when these blooms consist of the taxa *Microcystis*, *Anabaena*, or *Planktothrix* (Chorus and Bartram, 1999). MCs are chemically stable in water (Jones and Orr, 1994; Harada et al., 1996) and resistant to eukaryotic and many bacterial peptidases (Dierstein et al., 2001), but susceptible to breakdown by some aquatic bacteria found naturally in diverse water bodies (Jones et al., 1994). More than

Abbreviations: MCs, microcystins; MC-LR, microcystin-LR; T-RFLP, terminal restriction fragment length polymorphism; DOC, dissolved organic carbon; HPLC, high performance liquid chromatography; BSA, bovine serum albumin; TRF, terminal restriction fragment; OTU, operational taxonomic unit; AWCD, average well colour development; PCA, principal components analysis; ANOVA, analysis of variance; CVA, canonical variate analysis; RDA, redundancy analysis; MANOVA, multivariate analysis of variance.

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70 different MCs have been characterized and MC-LR has been the most studied due to its high toxicity and frequent production. Toxicity of MCs have been described for animals (Milutinović et al., 2003; Žegura et al., 2008) and plants (Mcelhiney et al., 2001), while their ability to affect microbial communities structure was shown by Christoffersen et al. (2002). The detrimental effects of MCs on a broad spectrum of living organisms and their effect on ecosystem functioning (Codd et al., 2005) requires adequate ways for screening toxicity and for evaluating water quality of exposed water bodies. A number of works has been published investigating biodegradation of MCs in freshwater (Jones and Orr, 1994; Cousins et al., 1996) and some reported a link between previous exposure to MCs and rate of degradation (Christoffersen et al., 2002; Edwards et al., 2008). However the mechanisms that dictate the relation between past exposure and degradation rate have not been elucidated. Microorganisms and in particular bacteria have been studied in a number of ways through history starting from observation with magnifying glass by Antony van Leeuwenhoek back in the 17th century (Van leeuwenhoek, 1702) to arrive to newly-developed high-output DNA sequencing (Gobet et al., 2012). Different methods measure different parameters (e.g. morphology, physiology, biochemistry, molecular biological structure and diversity) of the bacterial communities and some authors showed how different methods can lead to diverse results (Grayston et al., 2004; Singh et al., 2006). Here we used T-RFLP to evaluate bacterial communities' structure and Biolog EcoPlate to determine their physiology. We investigated the relation between bacterioplankton communities structure and physiology of six Scottish water bodies previously studied by Edwards et al. (2008) and: a) past exposure to MCs; b) half-life of MC-LR; c) water chemical and physical parameters.

2. Material and methods

2.1. Sites and water sampling

Six Scottish water bodies were selected to include various cyanobacterial bloom exposure histories and MC-LR exposure. The water bodies chosen were Loch Rescobie (NO 525 515) and Loch Balgavies (NO 523 516) two closely located lakes with the outflow from the former flowing into the later via a small stream (approximately 600 m long) with both waterbodies supporting populations of microcystin producing cyanobacteria. Loch Forfar (NO 450 507) approximately 7 km to west of these lakes annually supports cyanobacterial blooms however microcystins have never been detected here. There is no direct water flow between any of these lakes. Loch Leven (NO 132 018) which is located 60 km south west of Forfar Loch and often supports cyanobacterial growth but microcystins have only been detected on a couple of occasions over a 20 year sampling period. River Carron (NO 877 857) and River Cowie (NO 876 864) are closely located rivers around 50 km north east of Forfar Loch and as fast-flowing rivers have no previous history of significant cyanobacterial growth and no microcystin occurrence. All water sources were also selected since they have been involved in a number of previous studies and their history of supporting cyanobacteria and microcystin was known (Personal observations). Details about cyanobacterial bloom history, MC-LR exposure and half-life of MC-LR in these water bodies are outlined in Table 1. Surface water samples were collected in triplicate on 26 September 2007 from the selected water bodies in sterile 1 l Duran glass bottles and stored at 4 °C over night until analysed. Surface water temperature and pH were measured at the site using a thermometer and pH metre (Jenway, Essex, UK). Water samples were filtered (0.45 μm cellulose acetate: Whaan, Kent, UK) and dissolved nitrogen (NO_3, NO_2, NH_4, and TN) and phosphate (PO_4 and TP) were determined colourimetrically using a San++ analyser (Skalar, Breda, the Netherlands). Dissolved organic nitrogen and phosphate were calculated as the difference between total and inorganic values. Dissolved organic carbon (DOC) was determined automatically at 550 nm following persulphate/UV digestion (Schreurs, 1978). Samples for T-RFLP were immediately filtered in aliquots of 1 l onto 0.22 μm pore size membrane filters (Millipore Stericup). The filters were removed in sterile conditions from the disposable filter units and stored in sterile Petri dishes at −20° C until DNA extraction.

2.2. Biodegradation of MC-LR

The half-life data of MC-LR were derived from the work of Edwards et al. (2008) for the same waterbodies and were analysed for statistical linkage between the microbial community structure and rate of degradation. In brief, water samples were prepared by placing 50 ml of freshly collected water in 100 ml sterile Erlenmeyer flasks stoppered with cotton wool bungs. MC-LR (final concentration of 1 μg ml^{-1}) was added aseptically in triplicate, to water samples and sterile control water samples (i.e. autoclaved). Incubation was at 29 °C shaking at 100 rpm. Aliquots (500 μl) for analysis were taken aseptically every 3–4 days, frozen, freeze-dried, reconstituted in 80% aqueous methanol and centrifuged at 15 000 × g then the supernatant analysed by HPLC (Edwards et al., 2008). The ability of microbial communities from the water bodies studied in this work to degrade MC-LR has been further investigated in other studies (Ghimire, 2007; Manage et al., 2009a) giving consistent results.

2.3. T-RFLP analysis

DNA was extracted from half of the polyethersulfone filter (Millipore Stericup) stored at −20 °C using the Power Soil DNA Extraction

Table 1
Water chemistry, MCs natural exposure and MC-LR half life for Scottish freshwater bodies under study. Water chemistry analysis done within 24 h of sampling.

	Loch Rescobie	Loch Forfar	Loch Balgavies	Loch Leven	River Carron	River Cowie
NH_4-N (μg ml^{-1})	0.46 ± 0.01	0.65 ± 0.04	0.17 ± 0.03	0.05 ± 0.00	0.08 ± 0.04	0.12 ± 0.06
NO_3-N (μg ml^{-1})	0.74 ± 0.00	2.22 ± 0.01	0.57 ± 0.00	0.07 ± 0.01	6.14 ± 0.08	1.12 ± 0.83
Total-N (μg ml^{-1})	1.75 ± 0.04	3.25 ± 0.06	1.36 ± 0.02	0.59 ± 0.03	6.33 ± 0.04	2.26 ± 0.13
Org-N (μg ml^{-1})	0.55 ± 0.09	0.37 ± 0.06	0.62 ± 0.01	0.48 ± 0.03	0.11 ± 0.10	0.53 ± 0.12
PO_4-P (μg ml^{-1})	0.13 ± 0.01	0.29 ± 0.02	0.22 ± 0.01	0.04 ± 0.06	0.04 ± 0.00	0.02 ± 0.01
Tot-P (μg ml^{-1})	0.15 ± 0.03	0.38 ± 0.05	0.29 ± 0.02	0.06 ± 0.06	0.04 ± 0.00	0.05 ± 0.03
Org-P (μg ml^{-1})	0.02 ± 0.01	0.09 ± 0.04	0.08 ± 0.02	0.02 ± 0.00	0.01 ± 0.01	0.03 ± 0.02
DOC (μg ml^{-1})	7.68 ± 0.28	5.83 ± 0.04	8.52 ± 0.33	6.76 ± 0.37	3.78 ± 0.53	9.09 ± 0.52
NO_2–N (μg ml^{-1})	0.03 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MCs exposure[a]	Regular	No	Regular	Occasional	No	No
pH	11.9 ± 0.00	7.4 ± 0.00	7.8 ± 0.00	8.5 ± 0.00	7.9 ± 0.00	7.8 ± 0.00
Temperature	11.1 ± 0.00	10.5 ± 0.00	11.6 ± 0.00	9.5 ± 0.00	8.6 ± 0.00	8.2 ± 0.00
MC-LR half-life (days) (source Edwards et al., 2008)	4	9	4	5	13	14

[a] Unpublished data from authors sampling over a period of >20 years. Regular = microcystins always found during typical bloom season (June–September), Occasional = microcystins only detected twice in >20 years, No = microcystins never detected in these locations (Lawton, Personal observations).