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Bacterial Degradation of Microcystin

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Abstract—Cyanobacteria exist under a variety of climatic, nutrient and physical conditions, and are likely to form blooms. This distinct group of bacteria is photosynthetic and produce several metabolites that include a number of endotoxins, of which are commonly found in mass occurrences of cyanobacteria, especially under eutrophic conditions. Microcystins (MCs) are well-studied cyanobacterial cyclic hepatopeptide heapatotoxins predominantly produced by freshwater cyanobacteria, including species of *Microcystis*, *Anabaena*, *Nostoc* and *Planktothrix*. Potential chronic toxicity from MC led the WHO to establish a guideline value of 1 μg l^{-1} as a maximum concentration of MC-LR in drinking water. Additional concern regarding the importance of cyanotoxins, is reflected by their inclusion in the US Environmental Protection Agency (USEPA) drinking water contaminant list and in major reviews along with chemical warfare agents. Furthermore, MC-LR was classified as a possible human carcinogen (group 2B). However, only very less data on the occurrence of microbial degradation of MC are available in the world.

Keywords: cyanobacteria, microcystin, bacteria, microbial degradation, toxicity

INTRODUCTION

Within recent years greater attention has been paid eutrophication of natural and artificial water bodies and production of toxic cyanobacteria blooms which produce cyanotoxins. It has become apparent that toxic cyanobacterial blooms are on the increase, presenting a hazard to animal and human health (Zurawell *et al.*, 2005). Among cyanotoxins, the stable cyclic structure of the peptide MCs has presented many challenges to water treatment facilities as conventional treatment methods have limited effect on the removal of MCs (Himberg *et al.*, 1989). Water treatment costs combined with water scarcity and increasing water demand present a huge problem in the developing world where populations are frequently exposed to cyanobacterial toxins amongst other organic and microbial contaminants. Thus, there is a need for simple, low cost and effective water treatment technology. Recently it has been documented that the use of slow sand filters and biofilms which exploit the use of selected biodegrading bacteria to complement the natural microbial flora of the filter for improved removal, providing a low cost solution for the provision of safe potable water (Babica *et al.*, 2005; Bourne *et al.*, 2006; Ho *et al.*, 2006, Edwards *et al.*, 2008). However, information is very limited on this regard. This paper aims to review some of the available information on microbial degradation of MC with specific objectives to provide preliminary information that has been already published on microbial degradation of a range of MCs, in a variety of water samples.

In situ studies on bioremediation of microcystin

The field studies conducted by Edwards *et al.* (2008) showed that significant differences in the rate of degradation and the half-life ($D_{1/2}$) of MC-LR in water collected from Loch Rescobie, Balgavies Loch, Loch Leven, Rivers Carron and River Cowie in Scotland, UK. It was showed that degradation rate of MCs were varying at each water body with previous bloom history where loch Rescobie and Balgavies frequently supported MC-containing blooms (Richard *et al.*, 1983), Loch Leven supports occasional MC-containing blooms (Edwards *et al.*, 2008), Forfar Loch, although eutrophic, has no record of toxic blooms and there have been no reports of blooms in the fast flowing Rivers Cowie and Carron. Those water bodies with no previous history of MC contamination, Forfar, Carron and Cowie demonstrated a notable lag period before degradation commenced. Also, previous published data have clearly indicated that past exposure to MCs results in considerably faster degradation rates in natural waters (Jones and Orr, 1994; Cousins *et al.*, 1996; Christoffersen *et al.*, 2002). Edwards *et al.* (2008) showed that the half-lives of MC-LR degradation in the Rivers Cowie and Carron were 14–15 d and are similar to the slower rates recorded in water from Finnish lakes with no previous occurrence of MC-producing blooms (Rapala *et al.*, 1994). This was further confirmed by analysis of natural loch water with the sterile controls monitored experiment. For example, no loss of MC-LR occurred in Rescobie water confirming the study period and the observed degradation was detected due to microbial populations present in raw water samples (Fig. 1).

BACTERIAL DEGRADATION OF MICROCYSTIN

Identification of bacteria that degrade MC have been reported from sewage effluent, lake and river water, lake sediment and infiltration soil areas of lake water (Sivonen and Jones, 1999; Holst *et al.*, 2003), but only a few bacterial strains with degradation ability towards MCs have been isolated. The majority of cyanotoxin biodegradation studies have focused on bacteria isolated from water sources exposed to MC containing blooms. The number and diversity of reported bacteria isolates have capable of MC degradation is still extremely low. Until recently the only bacteria characterized which were capable of degrading these cyclic peptides of the genus *Sphingomonas*. Rapala *et al.* (2005) described a novel bacterium, *Paucibacter toxinivorans* capable of degrading MCs and nodularin (NOD), and a MC-degrading bacteria strain Y2 was isolated in Japan and was classified as *Sphingosinicella microcystinivorans* (Maruyama *et al.*, 2006). The

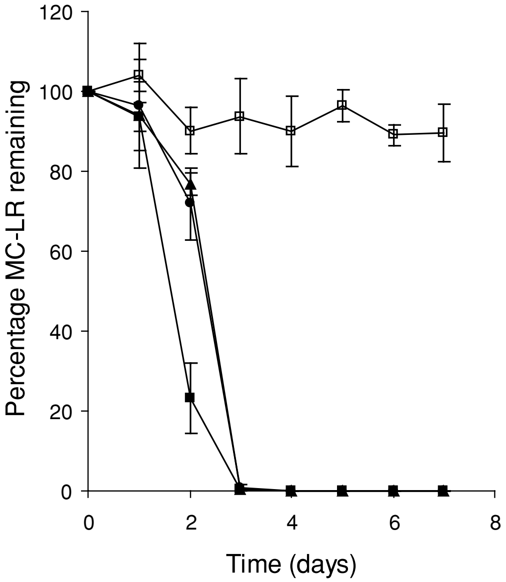

Fig. 1. Batch degradation of MC-LR (10 g ml^{-1}) by selected bacterial isolates in sterile Loch Rescobie water by isolates R1 (■), R4 (▲), R6 (●) and R9 (○) where sterile Loch water (□). Error bars represent one standard deviations (*n* = 3).

ability of those isolate to degrade other MCs and NOD were also has investigated and revealed that peptides with the Adda-Arg bond were successfully degraded whilst MC-LF, with Adda-F bond and 6(Z)-Adda MC-LR and 6(Z)-Adda MC-RR were not significantly degraded (Imanishi *et al.*, 2005). Another Japanese *Sphingomonas* isolate, 7CY, was shown to degrade a wider range of MCs, including MC-LR, MC-RR, MC-LY, MC-LW and MC-LF but it was unable to degrade NOD-Har (Ishii *et al.*, 2004). To date, Jones and Orr (1994), Bourne *et al.* (1996, 2001), Park *et al.* (2001), Saito *et al.* (2003), Harada *et al.* (2004) also have described the isolation of four *Sphingomonas* sp. or sphingomonad-like strains. Bourne *et al.* (1996), Takenaka and Watanabe (1997) have described other several bacterial strains involved in the degradation of MCs. Bourne *et al.* (1996, 2001) identified a gene cluster in *Sphingomonas* sp. ACM-3962 which was responsible for the degradation of MC-LR (Table 1).

Recently, degradation capability of MC-LR by novel bacteria isolated from Loch and river waters in Schotland, UK were recorded by Manage *et al.* (2009b). This study was based on the preliminary studies conducted to determine the degradation of MC by natural microbial community (Edwards *et al.*, 2008; Manage *et al.*, 2009a). Of 31 freshwater bacterial isolates, 10 were positive from loch Rescobie, Forfar loch and river Carron screened using the Biolog MT2 assay to determine their metabolism of the MC-LR. 16S rRNA phylogenetic analysis (16S rRNA) identified the novel bacteria as *Brevibacterium* sp., *Arthrobacter*

Table 1. Bacteria implicated in the degradation of microcystin toxins.

Bacterium	Source	Genebank accession number[a]	Reference(s)	Degradable analogous[b]	Non-degradable analogous[b]
Sphigomonas sp. ACM-3962	Urrumbidgee River, Australia	AF401172	Jones and Orr (1994) Bourne *et al*. (1996, 2001)	MCLR, MCRR	Nodularin
Sphigomonas sp. Y2	Lake Suwa, Japan	AB084247	Park *et al*. (2001) Maruyama *et al*. (2003, 2006)	MCLR, MCRR, MCYR, 6(Z)-Adda-MCLR	—
Sphigomonas sp. MD-1	Lake Kasumigaura, Japan	AB110635	Saito *et al*. (2003)	MCLR, MCRR, MCYR	Nodularin
Sphigomonas sp. B9	Lake Tsukui, Japan	AB159609	Harada *et al*. (2004) Imanishi *et al*. (2005)	MCLR, MCRR, 3-DMMCLR, DHMCLR, MCLR-Cys, Nodularin	MCLF, 6(Z)-Adda-MCLR, 6(Z)-Adda-MCRR
Sphigomonas sp. 7CY	Lake Suwa, Japan	AB76083	Ishii *et al*. (2004)	MCLR, MCRR, MCYR, MCLW, MCLF	Nodularin
Sphigomonas sp. MDB2	Tenryu River, Japan	AB219940	Maruyama *et al*. (2006)	—	—
Sphigomonas sp. MDB3	Tenryu River, Japan	AB219941	Maruyama *et al*. (2006)	—	—
Sphigomonas sp. CBA4	San Roque reservoir, Argentina	AY920497	Valeria *et al*. (2006)	MCRR	—
Sphigomonas witflariensis LH21	Myponga reservoir, Australia	DQ112242	Ho *et al*. (2007)	MCLR, MCLA	—

Bacterium	Source	Genebank accession number[a]	Reference(s)	Degradable analogous[b]	Non-degradable analogous[b]
Brevibacterium sp. F3	Loch Forfar, Scotland	FN392692	Manage *et al.* (2009b)	MCLR	—
*Arthrobacter*ia sp. C6	River Caron, Scotland	FN392690	Manage *et al.* (2009b)	MCLR	—
*Arthrobacter*ia sp. F10	Loch Forfar, Scotland	FN392691	Manage *et al.* (2009b)	MCLR	—
*Arthrobacter*ia sp. R1	Loch Rescobie, Scotland	FN392694	Manage *et al.* (2009b)	MCLR	—
*Arthrobacter*ia sp. R4	Loch Rescobie, Scotland	FN392695	Manage *et al.* (2009b)	MCLR	—
*Arthrobacter*ia sp. R9	Loch Rescobie, Scotland	FN392697	Manage *et al.* (2009b)	MCLR	—
*Arthrobacter*ia sp. R6	Loch Rescobie, Scotland	FN392696	Manage *et al.* (2009b)	MCLR	—
*Arthrobacter*ia sp. F7	Loch Rescobie, Scotland	FN392693	Manage *et al.* (2009b)	MCLR	—
Rhodococcus sp. C1	River Caron, Scotland	FN392688	Manage *et al.* (2009b)	MCLR	—
Rhodococcus sp. C3	River Caron, Scotland	FN392689	Manage *et al.* (2009b)	MCLR	—

[a] Genebank accession numbers for partial 16S rRNA gene sequences.
[b] Including nodularin.

spp., and *Rhodococcus* sp. that belong to the Actinobcateria (Manage *et al.*, 2009b). Those bacteria species were identified as MC-LR degraders and also were well known for their metabolic diversity and ability to degrade a range of natural and man-made chemical compounds (Ho *et al.*, 2007). Until recently, only members of genus *Sphingomonas* was reported to be able to degrade microcystin and the gene cluster responsible for microcystin degradation (*mlr*) has been reported for all *Proteobacteria* (Rapala *et al.*, 2005; Lemes *et al.*, 2008) where Manage *et al.* (2009b) recorded isolates belonged to the *Actinobacteria* and no PCR products specific for proteobacteria detected, whereas all target genes (*mlrA*, *mlrB*, *mlrC* and *mlrD*) produced PCR products in the positive control. Thus, the recent work possibly recorded new gene for MC degradation pathways (Manage *et al.*, 2009b).

By-products

Identification of biodegradation products of MC-LR observed in Edwards *et al.* (2008) was acyclo MC-LR (NH_2-Adda-Glu-Mdha-Ala-Leu-MeAsp-Arg-OH) with an *m*/*z* of 1013 and a characteristic fragmentation pattern indicative of the linear peptide as previously published (Bourne *et al.*, 1996) suggest that the microbes present degraded the MC-LR by cleavage of the Adda-R bond. Furthermore, during degradation of MC-LF and NOD in the Loch Rescobie water, new peaks were detected when the concentration of MC/NOD decreased. Demethylation of MC-RR by a *Sphingomonas* sp. isolate was recently reported in Argentina, representing the first report of a MC/NOD degradation compound produced via a route not involving sequential hydrolysis of specific peptide bonds. The current investigation on the intermediate breakdown products of NOD demonstrates the existence of multiple mechanisms (Edwards *et al.*, 2008).

The *in situ* finding of microbial degradation and *in vitro* records of MC-LR and other MCs degradation by bacterial studies clearly demonstrated that a greater diversity of bacterial genera can degrade MC-LR and the other MCs. The novel finding of Actinobacteria recorded by Manage *et al.* (2009b), revelled that uncharacterized degradation mechanism since no intermediate products were identified. Further studies are needed to elucidate the genes involved in MC degradation in the novel bacteria (Manage *et al.*, 2009b) and also practical applications of the microbes on water treatment processes as provide safe drinking will be a global challenge in near future with environmental pollution of freshwater bodies due to mass production of toxin producing cyanobacteria.

CONCLUSIONS

MCs are cyclic hepatopeptide hepatotoxins produced by several bloom forming cyanobacterial genera of *Microcystis*, *Anabaena*, *Oscillatoria*. One of the most commonly occurring MCs is the highly toxic microcystin-LR. MCs inhibit protein phosphatises and constitute a natural health hazards in the environment that has led to acute livestock and human poisoning. Furthermore, MC-LR has been shown to be tumour promoter in rats, and the presence of MCs in drinking water can be linked to an increased frequency of primary liver cancer among human. MCs are chemically stable in water and conventional water treatment processes such as coagulation, flocculation and filtration have failed to remove them to recommended levels required by the WHO. Thus, the effects of chronic toxicity from MC-LR have led the WHO to establish a guide line of 1.0 $\mu g\ l^{-1}$ as a maximum concentration of MC-LR in drinking water supplies. Therefore, to provide safe drinking water is a global challenge due to the occurrence of toxic cyanobacterial bloom. Many studies have reported biological degradation of microcystin in natural lakes and reservoirs. Thus, it seems one of the most exciting areas for a successful solution to remove cyanotoxin is harnessing microbes. Identification of bacteria that degrade microcystin have been reported from sewage effluent, lake and river water, lake sediment and infiltration soil areas of lake water, but only a few bacterial strains with degradable ability towards MCs have been isolated. Present paper described the occurrence, microbial degradation and isolation of bacteria involved in the degradation of MCs.

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