

Bacterial Degradation of Microcystin

Pathmalal M. MANAGE^{1,2}, Christine EDWARDS² and Linda A. LAWTON²

¹*Department of Zoology, University of Sri Jayewardenepura,
Gangodawila, Nugegoda, Sri Lanka*

²*School of Pharmacy and Life Sciences, The Robert Gordon University,
Aberdeen, U.K. AB25 1HG*

(Received 14 February 2010; accepted 4 March 2010)

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 heptapeptide hepatotoxins 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 $1 \mu\text{g l}^{-1}$ 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

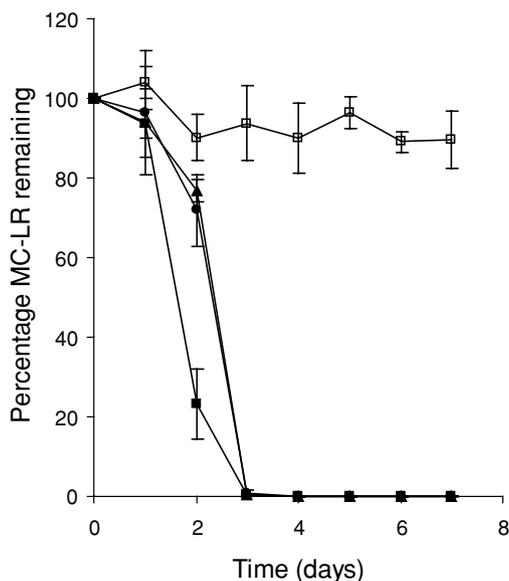


Fig. 1. Batch degradation of MC-LR (10 g ml^{-1}) by selected bacterial isolates in sterile Loch Rescobie water by isolates R1 (■), R4 (▲), R6 (●) and R9 (○) where sterile Loch water (□). Error bars represent one standard deviations ($n = 3$).

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 analogues ^b	Non-degradable analogues ^b
<i>Sphigomonas</i> sp. ACM-3962	Urrumbidgee River, Australia	AF401172	Jones and Orr (1994) Bourne <i>et al.</i> (1996, 2001)	MCLR, MCRR	Nodularin
<i>Sphigomonas</i> sp. Y2	Lake Suwa, Japan	AB084247	Park <i>et al.</i> (2001) Maruyama <i>et al.</i> (2003, 2006)	MCLR, MCRR, MCYR, 6(Z)-Adda-MCLR	—
<i>Sphigomonas</i> sp. MD-1	Lake Kasumigaura, Japan	AB110635	Saito <i>et al.</i> (2003)	MCLR, MCRR, MCYR	Nodularin
<i>Sphigomonas</i> sp. B9	Lake Tsukui, Japan	AB159609	Harada <i>et al.</i> (2004) Imanishi <i>et al.</i> (2005)	MCLR, MCRR, 3-DMMCLR, DHMCLR, MCLR-Cys, Nodularin	MCLF, 6(Z)-Adda-MCLR, 6(Z)-Adda-MCRR
<i>Sphigomonas</i> sp. 7CY	Lake Suwa, Japan	AB76083	Ishii <i>et al.</i> (2004)	MCLR, MCRR, MCYR, MCLW, MCLF	Nodularin
<i>Sphigomonas</i> sp. MDB2	Tenryu River, Japan	AB219940	Maruyama <i>et al.</i> (2006)	—	—
<i>Sphigomonas</i> sp. MDB3	Tenryu River, Japan	AB219941	Maruyama <i>et al.</i> (2006)	—	—
<i>Sphigomonas</i> sp. CBA4	San Roque reservoir, Argentina	AY920497	Valeria <i>et al.</i> (2006)	MCRR	—
<i>Sphigomonas wiffliarzensis</i> LH21	Myponga reservoir, Australia	DQ112242	Ho <i>et al.</i> (2007)	MCLR, MCLA	—

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

^aGenebank accession numbers for partial 16S rRNA gene sequences.

^bIncluding nodularin.

spp., and *Rhodococcus* sp. that belong to the Actinobacteria (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 (NH₂-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), revealed 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 heptapeptide 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 phosphatases 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 $\mu\text{g l}^{-1}$ 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.

REFERENCES

- Babica, P., L. Bláha and B. Marsálek (2005): Removal of microcystins by phototrophic biofilms. *Environ. Sci. Pollut. Res.*, **12**, 369–374.
- Bourne, D. G., G. J. Jones, R. L. Blakeley, A. Jones, A. P. Negri and P. Riddles (1996): Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin LR. *Appl. Environ. Microbiol.*, **62**, 4086–4094.
- Bourne, D. G., P. Riddles, G. J. Jones, W. Smith and R. L. Blakeley (2001): Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin-LR. *Environ. Toxicol.*, **16**, 523–534.
- Bourne, D. G., R. L. Blakeley, P. Riddles and G. J. Jones (2006): Biodegradation of the cyanobacterial toxin microcystin LR in natural water and biologically active slow sand filters. *Water Res.*, **40**, 1294–1302.
- Christoffersen, K., S. Lyck and A. Winding (2002): Microbial activity and bacterial community structure during degradation of microcystins. *Aquat. Microb. Ecol.*, **27**, 125–136.
- Cousins, I. T., D. J. Bealing, H. A. James and A. Sutton (1996): Biodegradation of microcystin-LR by indigenous mixed bacterial populations. *Water Res.*, **30**, 481–485.
- Edwards, C., D. Graham, N. Fowler and L. A. Lawton (2008): Biodegradation of microcystins and nodularin in freshwaters. *Chemosphere*, **73**, 1315–1321.
- Harada, K. I., S. Imanishi, H. Kato, M. Masayoshi, E. Ito and K. Tsuji (2004): Isolation of Adda from Microcystin-LR by microbial degradation. *Toxicol.*, **44**(1), 107–109.
- Himberg, K., A. M. Keijola, L. Hiisvirta, H. Pyysalo and K. Sivonen (1989): The effect of water treatment processes on the removal of hepatotoxins from *Microcystis* and *Oscillatoria* cyanobacteria: a laboratory study. *Water Res.*, **23**, 979–984.
- Ho, L., T. Meyn, A. Keegan, D. Hoefel, J. Brookes, C. P. Saint and G. Newcombe (2006): Bacterial degradation of microcystin toxins within a biologically active sand filter. *Water Res.*, **40**, 768–774.
- Ho, L., D. Hoefel, C. P. Saint and G. Newcombe (2007): Isolation and identification of a novel microcystin-degrading bacterium from a biological sand filter. *Water Res.*, **41**, 4685–4695.
- Holst, T., N. O. G. Jorgensen, C. Jorgensen and A. Johansen (2003): Degradation of microcystin in sediments at oxic and anoxic denitrifying conditions. *Water Res.*, **37**, 4748–4760.
- Imanishi, S., H. Kato, M. Mizuno, K. Tsuji and K. I. Harada (2005): Bacterial degradation of microcystins and nodularin. *Chem. Res. Toxicol.*, **18**, 591–598.

- Ishii, H., M. Nishijima and T. Abe (2004): Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. *Water Res.*, **38**, 2667–2676.
- Jones, G. J. and P. T. Orr (1994): Release and degradation of microcystin following algicide treatment of a *Microcystis aeruginosa* bloom in a recreational lake, as 248 determined by HPLC and protein phosphatase inhibition assay. *Water Res.*, **28**, 871–876.
- Lemes, G. A. F., R. Kersanach, L. da S. Pinto, O. A. Dellagostin, J. S. Yunes and A. Matthiensen (2008): Biodegradation of microcystins by aquatic *Burkholderia* sp. From a south Brazilian coastal lagoon. *Ecotoxicol. Environ. Safety*, **69**, 358–365.
- Manage, P. M., C. Edwards and L. A. Lawton (2009a): Biodegradation of Microcystin-LR by Natural Bacterial Populations. p. 277–285. In *Interdisciplinary Studies on Environmental Chemistry—Environmental Research in Asia for Establishing a Scientist's Network*, ed. by Y. Obayashi, T. Isobe, A. Subramanian, S. Suzuki and S. Tanabe, TERRAPUB, Tokyo, 342 pp.
- Manage, P. M., C. Edwards, B. K. Singh and L. A. Lawton (2009b): Isoation and identification of Novel Microcystin Degrading Bacteria. *Appl. Environ. Microbiol.*, **75**(21), 6924–6928.
- Maruyama, T., K. Kato, A. Yokoyama, T. Tanaka, A. Hiraishi and H. D. Park (2003): Dynamics of microcystin-degrading bacteria in mucilage of *Microcystis*. *Microb. Ecol.*, **46**, 279–288.
- Maruyama, T., H. D. Park, K. Ozawa, Y. Tanaka, T. Sumino, K. Hamana, A. Hirashi and K. Kato (2006): *Sphingosinicella microcystinivorans* gen. nov., sp. nov., a microcystin degrading bacterium. *Int. J. Syst. Evol. Microbiol.*, **56**, 85–89.
- Park, H. D., Y. Sasaki, T. Maruyama, E. Yanagisawa, A. Hiraishi and K. Kato (2001): Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a heterotrophic lake. *Environ. Toxicol.*, **16**, 337–343.
- Rapala, J., K. Lahti, K. Sivonen and S. I. Niemelä (1994): Biodegradability and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. *Let. Appl. Microbiol.*, **19**, 423–428.
- Rapala, J., K. A. Berg, C. Lyra, R. M. Niemi, W. Manz, S. Suomalainen, L. Paulin and K. Lahti (2005): *Paucibacter toxinivorans* gen. nov., sp. nov., a bacterium that degrades cyclic cyanobacterial hepatotoxins microcystins and nodularins. *Int. J. Syst. Evol. Microbiol.*, **55**, 1563–1568.
- Richard, D. S., K. A. Beattie and G. A. Codd (1983): Toxicity of cyanobacterial blooms from Scottish freshwaters. *Environ. Technol. Lett.*, **4**, 377–382.
- Saito, T., N. Sugiura, T. Itayama, Y. Inamori and M. J. Matsumura (2003): Degradation characteristics of microcystins by isolated bacteria from lake Kasumigaura. *Water SRT-Aqua.*, **52**, 13–18.
- Sivonen, K. and G. Jones (1999): Cyanobacterial toxins. p. 41–111. In *Toxic Cyanobacterial in Water: A Guide to Their Public Health Consequences, Monitoring, and Management*, Chapter 3, ed. by I. Chorus and J. Bartram, E & FN Spon, London and New York, 416 pp.
- Takenaka, S. and M. F. Watanabe (1997): Microcystin-LR degradation by *Pseudomonas aeruginosa* alkaline protease. *Chemosphere*, **34**, 749–757.
- Valeria, A. M., E. J. Ricardo, P. Stephan and W. D. Alberto (2006): Degradation of microcystin-RR by *Sphingomonas* sp. CBA4 isolated from San Roque reservoir (Cordoba-Argentina). *Biodegradation*, **17**, 447–455.
- Zurawell, R. W., H. Chen, J. M. Burke and E. E. Prepas (2005): Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. *J. Toxicol. Environ. Health*, **8**, 1–37.