Title: Still challenging: The ecological function of the cyanobacterial toxin microcystin – What we know so far

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Microcystins (MCs) are the most commonly studied cyanotoxins. While these past studies have mainly focused on the toxicity of MCs, the evolutionary history of life has shown that toxicity can be considered as an assigned role to MCs. Nowadays, there is a growing interest in understanding the importance of cyanotoxins in any of the physiological processes or beyond at the ecological level. This review evaluates the variously proposed intracellular and extracellular functions of MCs and how they benefit the producing cyanobacterium. However, the strain-specific and divergent laboratory and field results obtained to date have made it difficult to generalize.

Keywords: Cyanobacteria, cyanotoxins, microcystins, physiological function

1. Introduction

Cyanobacteria, commonly known as blue green algae, are the first photosynthetic organisms that released oxygen to the atmosphere 2.32 - 2.45 billion years ago during the great oxygenation event (Rasmussen *et al.* 2008, Büdel 2011, Schirrmeister *et al.* 2011). Cyanobacteria are found almost in every imaginable habitat on earth from the ice fields such as the polar and alpine regions to hot springs and deserts (Ward *et al.* 1998, Sompong *et al.* 2005, Singh and Elster 2007, Zakhia *et al.* 2008, Büdel 2011, Bhaya *et al.* 2012, Whitton 2012, Castenholz 2015). Under favorable conditions, cyanobacteria form highly toxic blooms which have undesirable effects on humans, animals, and aquatic life (Ressom *et al.* 1994, Falconer and Humpage 2005, Falconer 2008, Smith *et al.* 2008, Stewart *et al.* 2008, Catherine *et al.* 2013, Zanchett and Oliveira-Filho 2013, Lévesque *et al.* 2014). These toxic blooms, often but not always occurring in eutrophic lakes, have been reported to occur frequently in many countries throughout the world (Sivonen and Jones 1999, Stewart *et al.* 2008, Paerl *et al.* 2011). Cyanobacterial toxicity is caused by a variety of secondary metabolites termed cyanotoxins which include a diverse group of organic compounds both from the chemical and toxicological points of view (Wiegand and Pflugmacher 2005). According to their chemical structures, they fall into three main groups including cyclic peptides, (microcystin and nodularin), heterocyclic compounds like alkaloids (anatoxin-a, anatoxin-a(s), saxitoxin, cylindrospermopsin, aplysiatoxins, lyngbyatoxin-a), and lipopolysaccharides (Kaebernick and Neilan 2001). Based on the biological effects on targets, they are classified into five groups; 1) the hepatotoxins, which include the microcystins (MCs) and nodularins, cause severe liver disruption by inhibition of protein phosphatase 1 and 2A in hepatocytes, 2) the neurotoxins, composed of anatoxin-a, anatoxin a-(s), and saxitoxin, interfere with the functioning of neuromuscular systems, 3) the cytotoxins include cylindrospermopsins and alkaloids, cause damages in the liver, kidneys, spleen, thymus, and heart by inhibition of protein synthesis, 4) the dermatotoxins, such as aplysiatoxins, debromoaplysiatoxins, and lyngbyatoxins cause dermatitis as inflammatory agents and protein kinase C activators, and lastly 5) the irritant toxins, such as endotoxins, which are lipopolysaccharides associated with outer membranes of cyanobacteria that elicit irritation and allergic responses in human and animal tissues and also cause gastroenteritis and inflammation (Codd 1994, Carmichael 2001, Codd *et al.* 2005, Wiegand and Pflugmacher 2005, Leflaive and Ten-Hage 2007, Falconer 2008, Smith *et al.* 2008, Bláha *et al.* 2009, Pearson *et al.* 2010, Zanchett and Oliveira-Filho 2013).

Among the cyanotoxins, MCs are the most commonly studied and was first isolated from *Microcystis aeruginosa*, the organism from which their name is derived. Although other cyanobacterial species such as *Anabaena*, *Planktothrix*, *Nostoc*, and some species of benthic *Oscillatoria* produce MCs, *M. aeruginosa* is the species most often identified in freshwater cyanobacterial harmful blooms (Carmichael 1992, 2001, Wiegand and Pflugmacher 2005, Zurawell *et al.* 2005, Leflaive and Ten-Hage 2007, Pearson *et al.* 2010).

MCs are cyclic hepatopeptides composed of seven amino acids with the general structure of cyclo D-Ala^1 – X^2 – D-MeAsp^3 – Z^4 – Adda^5 – D-Glu^6 – Mdha^7, in which X and Z are variable L-amino acids, D-MeAsp is D-erythro methylaspartic acid, Adda is a unique 3-amino-9-methoxy-2, 6,8-trimethyl-10-phenyl-deca-4,6-dienoicacid, and Mdha is N-methyldehydroalanine (Figure 1 A) (Van Apeldoorn *et al.* 2007). Currently, more than 100 different congeners of MCs have been identified (Niedermeyer 2014). The variants typically vary at the X and Z-amino acids positions, the presence or absence of methyl group on D-MeAsp and / or Mdha and the substations of other moieties (Rinehart *et al.* 1994, Sivonen 1996, Namikoshi *et al.* 1998). The most common MC congeners are MC-LR, MC-RR, and MC-YR, containing L-amino acids leucine (L), arginine (R) or tyrosine (Y) at the X position, respectively (Harada 1996). Among the MCs congeners, MC-LR is the best-studied variant because of its abundance and toxicity (Harada 1996, Pearson *et al.* 2010, Ufelmann *et al.* 2012, Yu *et al.* 2014). MC-LR is the most toxic variant followed by MC-YR and MC-RR (Yu *et al.* 2014). However, the limited information for the other congeners, possibly due to the focus on MC-LR or lacking analytical methods, might have resulted in the underestimation of the toxicity of these MC variants and consequently, accepting MC-LR as the most toxic of the congeners (Fischer *et al.* 2010, Pacheco *et al.* 2016).

MCs are synthesized non-ribosomally *via* a complex multifunctional enzyme, MC synthetase, which includes non-ribosomal peptide synthetases (NRPS), polyketide synthetase (PKS), tailoring enzymes and an ABC transporter (Nishizawa *et al.* 2000, Welker and Von Döhren 2006, Pearson *et al.* 2010, Kaplan *et al.* 2012). MC synthetase is encoded by a 55-kb *mcy* gene cluster composed of 10 bidirectional genes, called *mcy*A-J, with a bidirectional promoter region of 732-bp between *mcy*A and *mcy*D (Figure 1 B) (Tillett *et al.* 2000, Pearson *et al.* 2010, Kaplan *et al.* 2012).

Studies revealed that non-toxic strains had lost a partial or total part of the MC synthetase genes resulting in a sporadic distribution of the *mcy* gene cluster (Rantala *et al.* 2004, Christiansen

et al. 2008). On the other hand, MC-deficient mutants were generated by insertional inactivation of genes such as *mcy*A or *mcy*B and partial deletion of *mcy*H in the *mcy* gene cassette (Dittmann *et al.* 1997, Pearson *et al.* 2004). As a result, MC-lacking mutants are able to produce other non-ribosomal peptides but not the MC variants which nominate them as useful tools to study the functions of MCs. For instance, competition studies between toxic (MC-producing) and non-toxic (MC-deficient) strains of *M. aeruginosa* under various conditions have provided more information regarding the importance of MCs to the producing cyanobacterium.

Figure 1 here.

From a toxicological point of view, MCs are known as eukaryotic protein phosphatase inhibitors. They inhibit eukaryotic serine/threonine protein phosphatases types 1 and 2A by irreversible covalent binding to the thiol group of cysteines of the catalytic domain which leads to a hyperphosphorylation of cytoskeleton proteins resulting in severe liver disruption and death by hypovolemic shock (Runnegar *et al.* 1995, Pearson *et al.* 2010). Therefore, a vast amount of research has been focused on the harmful effects of MCs on humans, animals, and aquatic organisms, while their natural physiological and / or ecological functions remain unclear (Merel *et al.* 2013a, 2013b). There are so many open questions which need to be answered: Why do cyanobacteria produce toxins in the first place? What is the advantage of toxin production for the producer? MCs are N-rich and high-cost products; since producers pay a high energetic price for MC synthesis, they might be an effective factor in the cellular metabolism beyond their toxicity. In the present review, the suggested roles of MCs, including intracellular and extracellular functions with the focus on *M. aeruginosa,* will be discussed to clarify the significance of MCs to the producing organism (Table 1, Table 2, and Figure 1 A).

2. MCs intracellular functions

2.1 Photosynthesis and light adaptation

The involvement of MCs in photosynthesis and light adaptation were proposed as a result of studies which found that the MC production rate depended on the photon irradiancy (Utkilen and Gjølme 1992, Wiedner *et al.* 2003). In contrast, light quality did not have a significant effect on MC production (Utkilen and Gjølme 1992). It has been reported that a very low light intensity decreased

the production MCs, however, so did high light intensity (Van der Westhuizen and Eloff 1985). Under natural conditions, the toxicity at the bloom's surface was higher and decreased with an increase in depth, suggesting a link to the light penetration depth (Utkilen and Gjølme 1992). Under laboratory conditions, an increase in light intensity to the saturated level of between 2960 to 3700 lm m^{-2}, resulted in increased MC production under continuous culture. However, with a further increase in light intensity the MC concentration was decreased without any changes in the growth rate (Utkilen and Gjølme 1992, Kaebernick and Neilan 2001, Wiedner *et al.* 2003).

On the other hand, genetic studies indicated that the transcription of the *mcy* gene cluster and therefore MC production is regulated by light quantity (Renaud *et al.* 2011). Studies showed that a three-fold higher concentration of MC-LR was produced during the light period of light–dark cycle suggesting that MC production is regulated *via* circadian photosynthetic rhythm (do Carmo Bittencourt-Oliveira *et al.* 2005). From this, it can be inferred that MCs plausibly participate in the central metabolism although they are known as secondary metabolites (Straub *et al.* 2011).

Research investigating the relation between MC production rate and chlorophyll a content raised more evidence in support of the hypothesis that MCs participate in photosynthesis activity and affected the chromatic pigment contents (Lyck 2004). It was also established that MCs occurred in a constant ratio to Chl a (1:2 mol: mol) (Long *et al.* 2001). The MC content in cyanobacteria was directly related to the chlorophyll a quota but was reversely related to the growth rate (Van der Westhuizen and Eloff 1985, Debolis and Juneau 2010). Under light limited conditions, the *M. aeruginosa mcy*B-lacking mutant contained less photosynthetic pigments, β-carotene, zeaxanthin, echinenone, and chlorophyll a, but a greater ratio of phycocyanine to chlorophyll a compared to the wild type. On the other hand, the growth of the wild type and the mutant strain was similar, suggesting that MCs might play a role in the light harvesting processes (Hesse *et al.* 2001). Although, another study obtained conflicting results with the *mcy*-knockout mutant strain at different light intensities as no changes in the photosynthesis efficiency were observed, however, differences in the thylakoids membrane and irregular gas vacuoles were demonstrated (Dittmann *et al.* 1997).

The hypothesized involvement of MCs in photosynthesis was further supported by studies using an immunogold-labeling technique that disclosed that the thylakoids membrane is the most MC-occupied cell site followed by the nucleoplasmic area. Physically, more than two-thirds of MCs were attached to the thylakoids membranes (Shi *et al.* 1995, Young *et al.* 2005, 2008). Although, a

further study using the cryofixation/cryosectioning technique demonstrated that most of the MCs were localized in the nucleoplasmic area and intracellular inclusions such as carboxysomes and polyphosphate bodies, rather than thylakoids membranes and the cell wall (Gerbersdorf 2006). Under high light irradiation, the ratio of MCs in outer to inner cellular parts increased, and a higher percentage of MCs were found close to the thylakoids membrane suggesting the probable role of MCs in light adaptation (Gerbersdorf 2006). Moreover, the *M. aeruginosa mcy*A-knockout mutant has been found to be dominant under low light, with the toxic genotype *M. aeruginosa* PCC 7806 showing a greater fitness to high light suggesting that MCs play a role in protection against photooxidation (Phelan and Downing 2011). In contrast, another study showed that under both low and high light irradiation (1480 and 5920 lm m^{-2}, respectively) a mixed culture was dominated by the MC-producing strain *M. aeruginosa* UTCC 300 which further emphasized the importance of MCs in light adaptation (Renaud *et al.* 2011). Comparative proteomic studies also revealed two NADPH-dependent reductases, phycobiliproteins, and RuBisCo, which is a Calvin cycle enzyme, were expressed differently in the wild type and *mcy*B^- mutant of *M. aeruginosa* PCC 7806. Furthermore, MC-protein binding was significantly enhanced under high light (51800 lm m^{-2}) which was assumed to increase the protein stability and avoid redox changes (Zilliges *et al.* 2011). Therefore, the potential role of MCs in photooxidative protection under high light is an advantage for the organism (Gerbersdorf 2006, Phelan and Downing 2011). In another study with *M. aeruginosa* PCC7806 and its MC-deficient mutant, differences in metabolic responses between strains upon exposing to high light intensity (18500 lm m^{-2}) were observed. Trehalose and sucrose, two general stress markers, accumulated more in the mutant while carbon reserves such as glycolate accumulated faster in the wild type. Additionally, the photosynthesis rate and high molecular weight carbohydrate contents were greater in the wild type (Meissner *et al.* 2015).

2.2 Environmental adaptations

In addition to light, the links between MC quota and a variety of other environmental factors such as water temperature, pH, and nutrient availability (specifically nitrogen and phosphorus) were observed (Dai *et al.* 2016).

A correlation between temperature and MC production has been reported. It was found that increasing temperature to the optimal growth temperature enhanced toxicity. In contrast, temperatures exceeding 28°C led to a declined MCs quantity (Van der Westhuizen and Eloff 1985).

A recent study by Mowe *et al.*(2015) showed that elevated temperatures of 33°C led to an increase in total MC cell quota of tropical *Microcystis* species while further increase in temperature to 36°C resulted in declined MC content. Increased temperature has been shown to stimulate *Microcystis* blooms formation during warm months in summer (Liu *et al.* 2011, O'Neil *et al.* 2012). Recent studies revealed that increased temperature promoted the frequency of *Microcystis* toxic strains over non-toxic strains as well (Davis *et al.* 2009, Dziallas and Grossart 2011, Lehman *et al.* 2013, Yu *et al.* 2014). Consequently, global warming raised not only the possibility of cyanobacterial blooms formation but also caused more toxic blooms. Dziallas and Grossart (2011) reported that in Boulder Lake (USA), Hamburg Innenalster (Germany), and Lake Taihu (China) the ratio of toxic to non-toxic populations was raised with increased water temperature. A field study of four lakes in the northeast US showed that temperatures elevated by 4°C caused a noticeable shift toward more toxic blooms (Davis *et al.* 2009). A laboratory study also showed that increasing the temperature from 20 to 32°C led to a significant rise in the ratio of toxic vs. non-toxic strains of *M. aeruginosa* and the transcript levels of *mcy*D as well. Additionally, the presence of heterotrophic bacteria affected both quantity and quality of MCs in *M. aeruginosa* HUB W333 depending on the temperature. Raised temperature enhanced the proportion of MC-LR to MC-YR and led to a greater toxicity in a mixed culture (Dziallas and Grossart 2011). Raised temperature up to 36°C also resulted in an increase in the ratio of MC-LR to MC-RR produced by *M. ichthyoblabe* (Mowe *et al.* 2015).Moreover, the toxicity of *M. aeruginosa* was increased both at a pH higher or lower than the optimal growth pH value which was pH 9 (Van der Westhuizen and Eloff 1983).

Recent studies demonstrated a correlation between dissolved inorganic carbon (DIC) and the growth and MC production of *M. aeruginosa*. In a competitive study, the effect of low and high DIC (0.365 and 7.658 mmol l^{-1} $KHCO_3$) on *M. aeroginosa* toxic and non-toxic strains, FACHB 912 and FACHB 469, co-cultured with green algae *Chlamydomonas microsphaera* were investigated. The growth of *M. aeruginosa* toxic and non-toxic strains was negatively affected by DIC without any significant changes in the chlorophyll content; however, the photosynthesis efficiency and chlorophyll content of green algae decreased. The results proposed that *M. aeruginosa* might be more adapted to low DIC condition (Zhang *et al.* 2012). Increased dissolved inorganic carbon had an adverse effect on the frequency of toxic *Microcystis* and MCs concentration in Lake Chaohu, China as well (Yu *et al.* 2014). Deficiency of intracellular inorganic carbon resulted in an increase in MC production of *M. aeruginosa* PCC 7806. Moreover, the toxic

wild type contained greater chlorophyll a content and consequently displayed higher photosynthetic efficiency compared to the *mcy*B$^-$ mutant, suggesting a role of MCs in environmental adaptation (Jähnichen *et al.* 2007).

In another study, the toxic *Microcystis* sp. KLL strain MG-K isolated from Lake Kinneret, Israel severely inhibited the growth of its non-toxic laboratory emerged successor (strain MG-J). Additionally, during the *Microcystis* bloom season, an MC-producing strain placed in dialysis membrane in Lake Kinneret survived while the non-toxic spontaneous mutant lysed within 24 h. These results suggested that *Microcystis* toxic strains have an advantage under natural conditions (Schatz *et al.* 2005).

2.3 Protection against oxidative stress

Under stress-induced conditions which trigger reactive oxygen species generation, toxigenic *Microcystis* survive longer (Dziallas and Grossart 2011). In a comparative study investigating the effect of hydrogen peroxide exposure on both the toxic and non-toxic *M. aeruginosa* strains, the toxic strain showed a lesser decrease in its chlorophyll a content. Moreover, by increasing the temperature both strains' MC content and reactive oxygen species were elevated (Dziallas and Grossart 2011). Another study with two toxic *Microcystis* spp. isolated from Brazilian water bodies, disclosed that limiting the nutrients (nitrogen and phosphorus) as a form of stress-induction led to increased MC production and *mcy*D expression (Pimentel and Giani 2014). A recent proteomic study using continuous cultures of *M. aeruginosa* PCC 7806 showed that under iron depletion physiological changes such as reduction in chlorophyll a content besides enhancement in MC production. Increased intracellular and extracellular MCs in continuous culture of *M. aeruginosa* PCC 7806 under iron depletion provided more evidence of the assistance role of MCs in the better fitness of *Microcystis* under oxidative stress conditions (Yeung *et al.* 2016). In addition, *mcy* gene transcripts were enhanced under oxidative stress in various quantities i.e. 20% for *mcy*B, 270% for *mcy*A, 330% for *mcy*H, and 370% for *mcy*D (Straub *et al.* 2011).

Proteomics studies revealed the potential role of MCs in protection against oxidative stress as MC bound covalently to the cysteine residues of certain proteins *via* its N-methyl-dehydroalanine moiety (Dziallas and Grossart 2011, Zilliges *et al.* 2011, Kaplan *et al.* 2012). These proteins include phycobiliproteins, CpcB and ApcA, RuBisCo, glutathione reductase, and various hypothetical proteins that were expressed differentially in the wild type and mutant strain (Zilliges *et al.* 2011).

Under oxidative stress due to the iron depletion, MCs showed a greater tendency to the binding sites in thioredoxin-regulated proteins (Alexova *et al.* 2016). In *M. aeruginosa* PCC 7806 wild type grown under high light and iron deficiency or exposed to 10 µM hydrogen peroxide, MC-protein formation was stimulated. On the other hand, in cultures treated with a protease such as subtilisin under high light (51800 lm m^{-2}), the large subunit of RuBisCo was more stable in the wild type. It was assumed that MC attachment to proteins avoid the dimerization of cysteines and consequently caused a delay in conformational changes of proteins and enzymes inactivation (Zilliges *et al.* 2011). Thus, the increased protein stability led to more adaptation to the various stresses (Zilliges *et al.* 2011, Kaplan *et al.* 2012). Moreover, under high light, the decreased oxygenase function of RuBisCo protected the cells against photorespiration (Gerbersdorf 2006). On the other hand, current findings indicated MCs as additional radical scavengers, which might protect the cells against oxidative stress damage (Martin-Luna *et al.* 2006a, Zilliges *et al.* 2011). The ability of MCs to bind to metals such as zinc and cadmium also point to the possible role of the toxin in metal detoxification in metal-induced oxidative stresses (Dziallas and Grossart 2011).

2.4 Nutrient metabolism and storage

It has previously been shown that changes in the concentration of nutrients such as nitrogen, carbon, and phosphorus led to a change in the MC production rate indicating a possible relation between toxicity and primary cell metabolism (Oh *et al.* 2000).

Increased nitrogen concentration promoted MC production to the highest level, whereas nitrogen depletion led to a decrease in MCs synthesis (Holland and Kinnear 2013). These findings confirm a high dependency of the MC production rate on the nitrogen supply, also as it is such an N-rich molecule (Harke and Gobler 2013). Moreover, the binding site of NtcA, a global nitrogen regulator in cyanobacteria, was found in the promoter region of the *mcy* gene cassette include bidirectional *mcy*DA promoter, *mcy*E, *mcy*H, *mcy*G, and *mcy*J promoters (Kuniyoshi *et al.* 2011). Under nitrogen starvation, *ntc*A transcripts increased, and reversely *mcy* gene expressions decreased (Harke and Gobler 2013). Therefore, NtcA might regulate *Microcystis* toxicity (Harke and Gobler 2013, Pimentel and Giani 2014). It was shown that under nitrogen depletion, 2-oxoglutarates, a signal of the carbon/nitrogen balance, increased NtcA binding to the *mcy*A promoter leading to a decrease in *mcy* gene expression (Kuniyoshi *et al.* 2011). As a result, based on the carbon-nitrogen status, MCs synthesis can be regulated by NtcA as a repressor (Kuniyoshi *et al.* 2011, Harke and Gobler 2013).

In a comparative proteomics study, different isoforms of the same proteins were found in *M. aeruginosa* toxic and non-toxic strains. Moreover, proteins involved in carbon-nitrogen metabolism and redox balance were expressed in various quantities. Comparisons between toxic and non-toxic strains also showed Calvin cycle enzymes and proteins involved in glycolysis and respiration expressed in higher quantity in *M. aeruginosa* PCC 7820, a known toxic strain (Tonietto *et al.* 2012). Under nitrogen depletion, proteins involved in carbon and nitrogen metabolism such as chaperones and proteases were accumulated more in the non-toxic *M. aeruginosa* strains, PCC 7005 and *mcy*H$^-$ mutant (Alexova *et al.* 2016).

On the other hand, a study using immunogold labeling revealed MCs are generally localized within carboxysomes suggesting a possible relation between MCs synthesis and carbon fixation (Gerbersdorf 2006). Studies with *M. aeruginosa* PCC 7806 wild type and its *mcy*B$^-$ mutant cultured under inorganic carbon deficiency showed that the intracellular and extracellular quota of MCs had been elevated. Moreover, in the wild type, the concentration of chlorophyll a was higher. Consequently, *M. aeruginosa* PCC 7806 could adapt to the C-limited conditions better than the non-toxic mutant strain (Jähnichen *et al.* 2007). It was elucidated that MCs act as a RuBisCo inhibitor to better adapt to carbon fluctuations (Gerbersdorf 2006, Jähnichen *et al.* 2007, Dziallas and Grossart 2011). Under inorganic carbon limitation, which occurs in dense blooms by inhibition of the RuBisCo synthesis, the oxygenase function of RuBisCo decreased leading to lower CO_2 consumption, decreased carbon leakage by photorespiration and, consequently retaining the CO_2/O_2 ratio at a higher level (Jähnichen *et al.* 2001, 2007).

MCs were also found in the vicinity of polyphosphate inclusion bodies which are known as phosphate storage granules and metal trapping area (Gerbersdorf 2006). Enhancing the concentration of phosphorus led to an increase in the ratio of toxic to the non-toxic population (Davis *et al.* 2009, Yu *et al.* 2014). Increased MCs beside a lower C-fixation rate in *M. aeruginosa* UTEX 2388 under P-limited conditions were observed (Oh *et al.* 2000). Under severe P-limitation, the ratio of MC-LR to MC-RR was increased leading to shifting toward the more toxic variant MC-LR. The MC content was greater while the carbon fixation rate and as a result growth was lower (Oh *et al.* 2000).

Concerning MC's tendency to form complexes with metals such as zinc and cadmium, a possible role of MCs in detoxification or metal storage has been proposed (Young *et al.* 2008).

Whether the intracellular function of MCs is multifarious and multifaceted or just not fully elucidated yet remains unknown, however, of all currently proposed roles (Table 1) each holds some merit in its own right.

Table 1 here.

3. MCs extracellular functions

3.1 Quorum sensing (cell - cell communication)

Many bacterial species produce signal molecules such as acylated homoserine lactones and oligo-peptides to synchronize cellular activities in response to the environmental changes, a process called "quorum sensing" (Miller and Bassler 2001). Do MCs play any role as infochemicals in intercellular communications? To have this extracellular function, first MCs must be secreted from the cells to the environment. MCs are produced continuously from the early logarithmic phase to the late stationary phase (Lyck 2004). During growth and under different environmental conditions, MCs are exported partly to the surrounding media as an extracellular toxin. Therefore, MCs are released into the extracellular environment which is a known character of a signaling molecule (Hotto 2007). Increased extracellular MCs under iron limitation in continuous cultures of *M. aeruginosa* PCC 7806 proposed the probable role of MCs as infochemical to enhance toxin production and consequently increase the fitness of *Microcystis* blooms (Yeung *et al.* 2016).

The hypothesis of MCs involvement in signaling processes was also supported by the discovery of a protein with homology to the ABC transporter that is encoded by a part of MCs synthesis gene cluster, *mcy*H. Thus, McyH might be involved in MCs export (Pearson *et al.* 2004). However, by inactivation of *mcy*H gene in the Δ*mcy*H mutant of *M. aeruginosa*, MC synthetase has not been detected, and consequently, MC synthesis was completely blocked. From this, it was proven that MC export is linked to MC production (Pearson *et al.* 2004). Another hypothesis proposed that McyH might play a role in the stability of MC synthetase or act as a sensor of MCs in *Microcystis* cell membranes; however, this remains unclear (Pearson *et al.* 2004, Kaplan *et al.* 2012).

Since quorum sensing is a cell density-dependent mechanism, it is essential to know the effect of cell density on MC production rate and if a certain cell density is required to trigger toxin production. The study with different initial cell numbers of *M. aeruginosa* inoculated to the Bold's

medium and unfiltered water from Lake Sinclair (Georgia, USA) showed that the final cell density was positively related to the initial cell numbers. Therefore, *M. aeruginosa* proliferated in a cell density-dependent manner (Dunn and Manoylov 2016). In a mesocosm study, *Microcystis* cells collected in proximity were added to the mesocosm. Consequently, increase in the cell density from 5×10^5 cells ml^{-1} to 7×10^6 cells ml^{-1} resulted in a considerable increase in MC production from 0.1 to 1.38 pg $cell^{-1}$ over a period of 6 h (Wood *et al.* 2012). Though, the increase in cell numbers might be considered as a stress factor and therefore could trigger an increase in toxin production induced under oxygen deficiency or nutrient limitations. Besides, extracellular MC was not increased which might be due to the time required to start toxin export (Wood *et al.* 2012). A field study at the Lake Rotorua (South Island, New Zealand) with a dense bloom of toxic *Microcystis* demonstrated that an increase in the cell concentrations from 7×10^4 to 4×10^6 cells ml^{-1} coincided with significantly increased *mcy*E transcripts, and total and extracellular MC quota (Wood *et al.* 2011). As a result, MC synthesis depended on the *Microcystis* cell density (Wood *et al.* 2011, 2012). However, cultures started with different inoculum sizes, regardless of the number of initial cells, had the same MC production rate in the end (Orr and Jones 1998).

A further study clarified that addition of pure MC-LR or crude extracts caused an enhancement in the toxicity of resting cells. In *Microcystis* cultures exposed to the *Microcystis* crude extracts for 24 h, the *mcy*B transcripts enhanced 12-fold (Schatz *et al.* 2007). Moreover, MCs released after cell lysis due to mechanical treatments or environmental stresses caused an increase in toxin production in resting cells, a known character of an intercellular signal (Schatz *et al.* 2007). Genetic studies also provided more pieces of evidences to confirm this hypothesis. It was revealed that the sequence of the *mcy* gene cluster is similar to the quorum sensing genes of *Rhizobium leguminosarum* (Dittmann *et al.* 2001). Additionally, two light regulated proteins, MrpA and B, were found in *M. aeruginosa* PCC 7806 with the similarities to Rhi A and B proteins of *R. leguminosarum* (Dittmann *et al.* 2001). Rhi A and B are known as signal mediators and induced directly by N-acyl-homoserine lactones (AHLs) in a cell density-dependent manner (Rodelas *et al.* 1999). MrpA was expressed strongly in the wild type but not detected in a *mcy*B^- mutant (Dittmann *et al.* 2001). MC addition to pure cultures enhanced the transcription levels of these proteins, especially in the wild type. Since the MC quota is also regulated by light irradiancy, it was assumed that there is an indirect relation between these light-regulated proteins and MCs as an intercellular signaling molecule (Dittmann *et al.* 2001). However, these light regulated proteins were not found

in the toxic *M. aeruginosa* NIES-843 strain indicating that they are strain-specific proteins and not necessarily linked to the toxicity (Frangeul *et al.* 2008).

3.2 Benthic survival and recruitment processes

Microcystis has a biphasic life cycle, pelagic growth that proliferates as a planktonic form in the summer and benthic sedimentation that sinks and joins the sediments in the winter which is called overwintering (Reynolds *et al.* 1981, Takamura *et al.* 1984). The benthic population contains a mixture of toxic and non-toxic genotypes (Catherine *et al.* 2013). Recent studies showed that in sediments, toxic *Microcystis* cells could survive and preserve their toxic ability for an extended period up to several years (Ihle *et al.* 2005, Misson *et al.* 2012a, 2012b, Catherine *et al.* 2013, Torres and Adámek 2013). The study of the sediments taken from Lake Grangent (Loire, France) showed that *Microcystis* cells were present at the surface and the depth of sediments (Latour *et al.* 2007, Misson *et al.* 2012a, 2012b). Latour *et al.* (2007) studied *Microcystis* cells in the sediments of Grangent reservoir (Lior, France), at the surface (0-2 cm), depths of 25-35 cm (1.5-year- old colonies) and 70 cm (14-year-old colonies). The highest biomass of *Microcystis* cells was found in the 25-35 cm layer (2300 colonies ml sediments $^{-1}$ compared to 250 colonies ml sediments $^{-1}$ at the surface and 600 colonies ml sediments $^{-1}$ in 70 cm layer). Then, it was assumed that *Microcystis* cells could be accumulated and preserved in sediments. Although depth and the age of the sediments caused physiological changes as well as decreased cellular metabolites, MCs were detected at higher concentrations, e.g. 1 pg MC-LR cell^{-1} at the 30 cm sediment compared to 0.3 pg MC-LR cell^{-1} at the surface of the sediment (Latour *et al.* 2007). It was therefore assumed that MCs are not used as a nitrogen source and that it might play a role in sustaining *Microcystis* colonies. Moreover, the fermentation of endogenous glucose could provide the required energy to maintain the vegetative cells in deep sediments (Moezelaar and Stal 1997). Further studies demonstrated that MCs could be synthesized in the cells that were deeply buried in the benthic sediments even after several years of sedimentations (Lake Grangent, Loire, France) (Misson *et al.* 2012a). Moreover, the cellular MC quota varied with the age of sediments (Misson *et al.* 2012a, 2012b). MC content decreased as the duration of the benthic life stage increased (Misson *et al.* 2012a). It was reported that MC quota had been changed sharply in the first few months of sedimentation, first significantly increasing followed by a significant decrease, whereas it was reduced gradually in old sediments and stayed almost stable at a low level for up to 6 years (Misson *et al.* 2012b). The decline in MC

quotas in long term sedimentations could result from a decrease in MCs synthesis, MCs release from the old damaged cells, or MCs consumption as a nitrogen source (Latour *et al.* 2007, Jähnichen *et al.* 2008, Misson *et al.* 2012a). However, MCs were not used as cellular nitrogen supply (Latour *et al.* 2007).

The synthesis of MCs is a high energy cost process, and whether the toxic strains gain an advantage through MC production over non-toxic subpopulations is still in doubt. The continued presence and stability of MCs in the benthic phase in spite of the extreme environmental conditions of sediments such as low temperature, oxygen deficiency, and darkness, propose the hypothesis of MC's involvement in benthic survival (Misson *et al.* 2012a). It was suggested that MCs play a role in the maintenance of vegetative cells during the benthic phase under the oxidative stress caused by unfavorable conditions of sediments (Ihle *et al.* 2005, Latour *et al.* 2007, Zilliges *et al.* 2011, Misson *et al.* 2012b, Torres and Adámek 2013). It has been shown that MCs could be attached to the proteins under stress conditions to keep them active and preserve their functional structure (Zilliges *et al.* 2011, Misson *et al.* 2012b). Therefore, there might be a link between the ecological importance and the physiological functions of MCs.

In the life cycle of *Microcystis*, overwintering followed by the recruitment of cells from sediments to the water column in the spring is an important process to the annual succession of *Microcystis* (Reynolds *et al.* 1981, Takamura *et al.* 1984, Misson *et al.* 2011, Catherine *et al.* 2013). Although the recruitment is initiated in response to changing environmental factors, mainly increase in temperature and light penetration of water, the implication of MCs in benthic recruitment was proposed in recent laboratory and field studies (Ihle *et al.* 2005, Schöne *et al.* 2010, Misson *et al.* 2012a). Laboratory studies showed that reinvasion was profoundly affected by light and temperature (Schöne *et al.* 2010, Misson *et al.* 2011). By increased temperature (from 4 to 8°C) under illuminated condition, different subpopulation left the sediment and re-entered the water column (Misson *et al.* 2011). Increased temperature and low light intensity that penetrates to the bottom of sediments promote the resumption of metabolic activity of the benthic *Microcystis* cells, resulting in regaining the buoyancy of benthic colonies to migrate back into the water column (Reynolds *et al.* 1981, Tsujimura *et al.* 2000, Tan *et al.* 2008, Schöne *et al.* 2010).

The study of the sediments of Lake Quitzdorf (Germany) showed a preferred recruitment of MC-producing *Microcystis* to non-toxic strains under light (Schöne *et al.* 2010). During the recruitment process, the disappearance of *Microcystis viridis* (*mcy*B^+ genotype) from the sediments

was greater than *Microcystis wesenbergii* (*mcy*B$^-$ genotype). Moreover, up to 70% of the toxic genotype of *M. aeruginosa* were also re-entered from sediments to the water column (Schöne *et al.* 2010). Furthermore, studies of sediments from Lake Villerest (France) showed there was a preferential reinvasion selection related to the MC content and the colony size in the toxic subpopulations of *Microcystis* (Schöne *et al.* 2010, Misson *et al.* 2011). The smaller colonies (> 160 µm) with higher MC content (0.021 ± 0.004 pg eq MC-LR cell^{-1}) displayed a greater recruitment rate. Furthermore, the recruited *Microcystis* contained higher cellular quotas of MCs compared to the benthic cells. Regarding the unfavorable experimental conditions for MC production at the beginning of pelagic phase (darkness at 8°C), the preferential selection of more toxic population was proposed (Misson *et al.* 2011).

Moreover, during spring an annual decrease in *Microcystis* population in sediments was observed (Ihle *et al.* 2005). Field studies showed only a low number of cells (3-4%) were recruited successfully from sediments to the water column (Ihle *et al.* 2005, Schöne *et al.* 2010). During the recruitment process, cell lysis or programmed cell death was observed with an increase in extracellular MCs (Ihle *et al.* 2005). The addition of purified MC-LR (in the µg L^{-1} range) to the sediments led to a decrease in recruitment rates of both toxic (*mcy*B genotype) and non-toxic subpopulations that in the toxic subpopulation (25.7% and 6.4% reduction, respectively), with a greater decrease seen in the toxic subpopulation. Then, it was proposed that MCs play a role as an extracellular messenger (signal) in the regulation of reinvasion processes (Misson *et al.* 2012a).

3.3 Colony formation and bloom maintenance

Microcystis occurs in large colonies in nature but as unicellular forms in laboratory-grown cultures (Reynolds *et al.* 1981, Zhang *et al.* 2007, Sun *et al.* 2015). This colony formation characteristic assists *Microcystis* to form large blooms in nature, allowing it to dominate over other phytoplankton species, and migrate easier vertically in the water column to obtain the available nutrients (Bonnet and Poulin 2002, Gan *et al.* 2012, Yang and Kong 2012). Moreover, *Microcystis* in colonial form is more tolerant to the stress conditions (Wu *et al.* 2007). From this, the question as to whether MCs play a role in cellular aggregation arises. Continuous predator pressure by *Ochromonas* sp. as well as culturing with the filtered cultures of the flagellate fed with *M. aeruginosa* induced colony formation in unicellular *M. aeruginosa* PCC 7806 (Yang *et al.* 2009, Yang and Kong 2012).

The addition of MC-RR to *Microcystis* cultures caused a significant increase in extracellular polysaccharides that subsequently caused larger colonies to form. Therefore, extracellular MCs might be considered as an important factor in the maintenance of *Microcystis* blooms (Gan *et al.* 2012). A field study of *Microcystis* blooms in Lake Wannsee (Berlin, Germany) showed larger colonies (>100 mm) contained a greater proportion of toxic strains to non-toxic and also higher toxicity. Moreover, by increasing the colony size from the ratio of toxic to non-toxic genotypes, the concentration of MCs per cell was enhanced (Kurmayer *et al.* 2003).

Further studies regarding cellular surface exposed proteins involved in cellular interactions provided more evidence of the probable role of MCs in colony formation (Kehr *et al.* 2006, Zilliges *et al.* 2008). MCs were shown to bind covalently to a lectin microvirin protein which has a known role in cell to cell attachments. It is important to note that non-toxic strains contain different kinds of microvirin and that the microvirin deficient mutant produced lower MC quantities (Kehr *et al.* 2006). Thus, there might be a relation between microvirin, toxin production rate, and colony formation (Kehr *et al.* 2006).

On the other hand, a recent study revealed a relation between MCs and MrpC, a novel surface-exposed MC-related protein. MrpC is a strain-specific glycoprotein of *M. aeruginosa* strain PCC7806 that has a role in cellular aggregation (Zilliges *et al.* 2008). In the MC-deficient mutant *mcy*B$^-$, the MrpC quantity has been remarkably increased, and mutant cells showed a greater cellular aggregation compared to the wild type (Zilliges *et al.* 2008). Since the expression level of MrpC in the mutant was the same as wild type, MCs may affect the general stability of MrpC by interfering with the post-transitional modifications such as glycosylation status, or the expression level of other binding surface partners such as sugar binding proteins or lipopolysaccharides (Zilliges *et al.* 2008). The absence of glycosylation may lead to a protein inactivation or degradation that has been shown in other pathogenic bacteria like *Haemophilus influenza* (Grass *et al.* 2003). Cell surface-associated proteins, lectin microvirin and MrpC, were accumulated differentially in the wild type and MC-deficient mutant (Kehr *et al.* 2006, Zilliges *et al.* 2008). However, no evidence concerning the direct interactions of MCs with these proteins has been found.

3.4 Defense against zooplankton

Interactions between cyanobacteria and zooplankton are considered as aquatic predator–prey relations. Colony formation, lower long chain saturated fatty acids production, and increased

toxicity have been reported in *Microcystis* cultures exposed to grazers (Müller-Navarra *et al.* 2000, Jang *et al.* 2003, Watson 2003, Yang *et al.* 2006, Yang and Kong 2012).

Direct and indirect exposure of toxigenic *M. aeruginosa* strains (NIES 44, 87 and 88) to the zooplankton *Moina macrocopa*, *Daphnia magna,* and *Daphnia pulex* resulted in a significant increase and then decrease in MC production during six-day exposure experiments (Jang *et al.* 2003). The rate of increase depended on the growth stages, population densities, and concentrations of cellular exudates of the zooplankton. Compared to the juveniles and neonates, the adult zooplankton produced higher infochemicals that resulted in a greater increase in MC production (Jang *et al.* 2008). Additionally, higher cell density and a higher concentration of culture media filtrate of zooplankton led to a significant increase in both intracellular and extracellular toxin production by *Microcystis*. Results suggested that MC synthesis was triggered by infochemicals released from herbivorous zooplankton (Jang *et al.* 2007a). Moreover, colony formation was observed in *M. aeruginosa* exposed to the flagellate *Ochromonas* sp. as well (Yang *et al.* 2006, Yang and Kong 2012). Then, the increased MC production and colony formation were considered as efficient inducible defensive responses of *M. aeruginosa* to the grazing force of herbivorous zooplankton as exposure caused death in zooplankton (Rohrlack *et al.* 1999b, Jang *et al.* 2003, Jang *et al.* 2007a). Another study reported that *Daphnia galeata* fed with the wild type of *M. aeruginosa* PCC7806 died rapidly compared to the cultures exposed to the non-toxic mutant strain (Rohrlack *et al.* 1999b). Additionally, the toxic strain caused a decrease in the mobility of the daphnids. Thus, the toxigenic *Microcystis* can use the benefits of toxicity as an anti-predator defense mechanism. However, the ingestion rate of the wild type versus mutant strains was the same, meaning that the daphnids were not deterred by toxin production (Rohrlack *et al.* 1999b). In fact, filtering and feeding behavior of *Daphnia* spp. was not influenced by the presence or absence of MCs (Rohrlack *et al.* 1999a, 1999b). The inhibitory effects were related to the mechanical inhibition of maxillule movement and swallowing rate caused by the morphology of *Microcystis* such as the high viscosity of mucilaginous envelope and colony size of colony forming *Microcystis* that exceeded the size limit for food intake by the daphnids (Rohrlack *et al.* 1999a). Regarding the refuse of MC effects on *Daphnia* sp.'s ingestion rate, the significant reduction of food intake reported by unicellular toxic strain may be related to other factors which require further investigation (Rohrlack *et al.* 1999a, Müller-Navarra *et al.* 2000).

The diversity and distribution of *Daphnia* spp. were negatively correlated to the *Microcystis* blooms biomass (Reichwaldt *et al.* 2013). In a small eutrophic lake, the higher diversity and larger zooplankton biomass were observed at the sites of lower *Microcystis* bloom densities (Reichwaldt *et al.* 2013). Increased MC production in response to the direct and indirect exposures of *M. aeruginosa* toxic strains NIES 44, 88 and 99 to phytoplanktivorous (*Hypophthalmichthys molitrix*) and omnivorous fish (*Carassius gibelio langsdorfi*) provided more evidence in support of the inducible defensive role of MC which are triggered by the infochemicals, known as kairomones, released from fish (Jang *et al.* 2004, Ha *et al.* 2009). The increased concentrations of infochemicals induced increased MC production. Infochemicals produced by fish influenced MC production even though physical contact was not necessary (Ha *et al.* 2009).

Above all, cyanobacteria evolved a long time before the metazoans, and therefore defense against predators as the primary role of MCs is doubted, however; perhaps toxicity was introduced as a recent evolutionary role of MCs (Kaplan *et al.* 2012). Furthermore, the role of MCs as anti-herbivore chemical defenses is ambiguous. Further field studies showed that even high concentration of toxin is not enough to stop daphnid proliferation so that it might be used as a biological control of blooms (Chislock *et al.* 2013). Although the co-existence of zooplankton and toxic *Microcystis* in eutrophic habitats might be due to the reciprocal defenses that contain the induced toxin increase in *Microcystis* and induced tolerance in zooplankton (Jang *et al.* 2007a). Feeding inhibition of toxic *Microcystis* by chemosensory means (Copepods) or inhibitory consumption of large colonies (some small Cladocerans) and physiological resistance to MCs (*Brachionus calyciflorusor* and *Daphnia pulicaria*) were introduced as defense mechanisms in zooplankton (Fulton and Paerl 1987, DeMott *et al.* 1991).

3.5 Iron acquisition

Iron is a vital growth factor involved in photosynthesis, respiration, nitrogen fixation, and detoxification of oxygen radicals (Sunda 2001). MC production rate has been reported to change with different concentrations of iron (Sevilla *et al.* 2008, Zakhia *et al.* 2008). Under low iron level (below 2.5 µmol L^{-1}), the cellular growth was slower, but the toxicity was 20 – 40% higher (Lukač and Aegerter 1993). Increased MCs content under iron depletion (10 nmol l^{-1}) together with a greater accumulation of phycobilisome proteins and FutA, the ferric iron transporter, were also observed in toxic *M. aeruginosa* PCC 7806 strain, but not in non-toxic *mcy*H^{-} mutant and PCC

7005 strains (Alexova *et al.* 2011, 2016). Moreover, at severe and long-term iron limitations, MC-producing strain survived longer and had a greater iron uptake compared to non-toxic strains, and the toxin production rate was found to be higher (Martin-Luna *et al.* 2006b, Alexova *et al.* 2011). Therefore, it was proposed that MCs perhaps serve as a siderophore especially during extended iron starvation or act as an iron storage component (Martin-Luna *et al.* 2006b, Alexova *et al.* 2011). However, increased toxicity under iron depletion might be considered as a response to this environmental stress as well.

It was shown that under low concentration of Fe (III), *M. aeruginosa* PCC7806 released superoxide to the extracellular environment that converts Fe (III) to Fe (II) which was later taken up through a siderophore-mediated system (Fujii *et al.* 2010). However, regarding low affinity of MCs to ferric iron which is inconsistent with the properties of siderophore, it was suggested that MCs might act as a shuttle to transfer iron through the cellular membrane (Klein *et al.* 2013).
Supplementary evidence of the relation between toxicity and iron uptake was obtained by finding the binding site of Fur in the promoter region of *mcy* gene cassette between *mcy*A and *mcy*D (Martin-Luna *et al.* 2006a, Kaplan *et al.* 2012). Fur, a ferric uptake regulator, is known as an iron availability and oxidative stress antenna in prokaryotes (Whitton 2012). It is involved in iron homeostasis, regulation of the genes responsible for oxidative stress responses, and cellular metabolism (Martin-Luna *et al.* 2006b). At sufficient concentration of iron; binding of Fur to the regulator region of DNA suppresses the expression of these genes (Whitton 2012). Under iron starvation, Fur was expressed 2-fold higher alongside an increase in the MC production rate (Martin-Luna *et al.* 2006a, 2006b, Whitton 2012). Thus, Fur is involved in the regulation of iron uptake and MC biosynthesis (Dittmann *et al.* 1997, Martin-Luna *et al.* 2006a, Alexova *et al.* 2011, Whitton 2012). On the other hand, the iron uptake capacity of cells at high light has been shown to be enhanced (Kaebernick and Neilan 2001). Light intensity controls both iron uptake and MC biosynthesis. Moreover, reduction of Fe (III) resulting from photoreduction or by superoxide dismutase made iron more available for the organism, which was later taken up by an iron-transfer system (Fujii *et al.* 2010). Thus, the possibility of a light regulating system was proposed (Whitton 2012).

3.6 Allelopathic interspecies interactions

Cyanobacteria are important members of the phytoplankton community which also contains other organisms such as diatoms, dinoflagellates, as well as green, red and brown algae. In aquatic habitats, cyanobacteria dominate the algal communities at the different seasonal cycle in a successive wave (El Herry *et al.* 2008). Previous publications showed that not only the environmental physical factors but also the interspecies biological interactions known as allelopathy, influence the algal succession and bloom formation (Legrand *et al.* 2003, Figueredo *et al.* 2007, Zhang *et al.* 2015). The production of allelopathic compounds has been observed in a wide variety of phytoplankton species (Lewis Jr 1986, Legrand *et al.* 2003, Gantar *et al.* 2008). In several studies, it has been shown that toxins can be considered as allelochemical (Rengefors and Legrand 2001, Graneli *et al.* 2008, Jaiswal *et al.* 2008). The producer can use this benefit to outcompete the other algal species in aquatic ecosystems (Rengefors and Legrand 2001, Legrand *et al.* 2003, Graneli *et al.* 2008). The interactions between *M. aeruginosa* and other cyanobacteria, other members of phytoplankton community, and aquatic plants can be taken into account as allelopathy. MCs affect the target species by photosynthesis inhibition, growth inhibition, and oxidative stress induction (Legrand *et al.* 2003, Gantar *et al.* 2008).

Allelopathy was proposed as an essential tool of cyanobacteria to outcompete diatomic population in a eutrophic lake (Keating 1978). The results were obtained by measuring the growth rate in mono and mixed cultures (Leão *et al.* 2009). The allelopathic function of MCs was further observed as the growth inhibition of various algal species, such as *Chlamydomonas, Haematococcus, Navicula and Cryptomonas*, and cyanobacteria with exposure to *Microcystis* and MC-LR (Kaebernick and Neilan 2001, Singh *et al.* 2001, Babica *et al.* 2006, Leão *et al.* 2009). However, the growth and photosynthesis of the dinoflagellate *Peridinium gatunense* grown in the cell-free filtrate of *Microcystis* sp. (KLL strain MB) were severely inhibited; exposure to pure MC-LR only resulted in slight effects. It was later proposed that cellular extracts include some metabolites that enhance the effects of produced toxins (Sukenik *et al.* 2002). In another study, exposure of different aquatic organisms to the crude extract of a cyanobacterial bloom isolated from Lake Müggelsee (Berlin, Germany) caused an elevation in the activity of the detoxification enzymes and, moreover, led to photosynthesis inhibition in *Scenedesmus armatus* and *Ceratophyllum demersum*. However, the toxicity of the crude extract was remarkably higher than the concentration of pure MC used (Pietsch *et al.* 2001). The inhibitory effects of MC-LR on the growth and photosynthesis of aquatic macrophytes *C. demersum* and *Myriophyllum spicatum* were

observed at the environmentally relevant concentration of 5 µg L^{-1} (Pflugmacher 2002). The photosynthetic pigment composition changed and the chlorophyll contents reduced (higher ratio of Chl b to Chl a) (Pflugmacher 2002). Additionally, the antioxidative enzymes of *C. demersum*, superoxide dismutase, glutathione peroxidase, ascorbate peroxidase, and dehydroascorbate reductase, were induced indicating oxidative stress as a result of MC exposure (Pflugmacher 2004). In another study, *Medicago sativa* was exposed to 5.0 µg $L^{-1}$of MC-LR and cyanobacterial bloom extract that led to the oxidative stress induction, lipid peroxidation and elevation of antioxidative enzymes, germination inhibition, and a decrease in the root length of alfalfa seedlings (Pflugmacher *et al.* 2006). Short time exposure of *Cyprinus carpio L* to MC-LR-induced oxidative stress mediated hydroxyl radicals in the carp (Jiang *et al.* 2013). The allelopathic interaction between duckweed *(Lemna japonica Landolt*) and toxic *M. aeruginosa* strains resulted in MC concentration increase and growth inhibition in the cyanobacterium and decrease in the growth of the aquatic plant (Jang *et al.* 2007b). By increasing the initial density of *Microcystis* cells in a co - cultivation of *M. aeruginosa* and *Chlorella* sp., the growth of green algae was changed from stimulation to the growth inhibition (Hong *et al.* 2010).

Concerning allelopathic studies, most investigations include two main procedures; exposing the target species to the pure MC as well as to *Microcystis* crude extract and culturing of target species with the culture media filtrates of *Microcystis* strains. Besides the cellular exudates and pure toxin, studies of co - cultivation systems and under continues toxin exposure to mimic the natural ecosystems can provide more practical information. The interspecies interactions of two MC-producing species *M. aeruginosa* CPCC 299 and *Planktothrix agardhii* NIVA-CYA 126 revealed that the presence of competing cyanobacteria affected the growth, *mcyE* gene copies, and McyE transcripts of both species negatively in mixed cultures compared to monocultures (Ngwa *et al.* 2014).

The presence of a diverse group of heterotrophic microorganisms associated with cyanobacterial blooms was reported in the field studies (Eiler and Bertilsson 2004, Kolmonen *et al.* 2004). Interspecies interactions with heterotrophic bacteria influenced MC production (Dziallas and Grossart 2011). Exposure of toxic strain of *M. aeruginosa* PCC 7806 and its mutant to hydrogen peroxide led to a decrease in MCs content. This decline was higher in axenic cultures compared to the xenic cultures containing heterotrophic bacteria added from a nutrient-poor Lake Stechlin (Germany) or the nutrient-rich Lake Dagow (Germany) indicating the contribution of heterotrophic

bacteria to radical scavenging. Moreover, with increased temperature, the MC production in the xenic cultures was significantly greater than the axenic culture (Dziallas and Grossart 2011).

Table 2 here.

Collectively, the proposed extracellular functions (Table 2) might clarify the ecological importance of MCs in the dominance of toxic bloom-forming *Microcystis*. MCs might be considered as an assistant factor that in combination with other environmental factors helps *Microcystis* to outcompete the other co-existing organisms and form blooms in a successive wave.

4. Future outlook and concluding remark

M. aeruginosa, the most common freshwater bloom-forming cyanobacterium, possess a rich reservoir of strain-specific flexible genes that support its evolutionary ecological adaptations and success in bloom formation (Humbert *et al.* 2013). A large number of genes encode secondary metabolites containing MCs (Humbert *et al.* 2013). MCs are the most commonly studied cyanotoxins in the environment. Although as research to date mainly focusing on the toxicity of MC, the possible primary functions of MCs have not yet been clarified. However, there is a growing interest in understanding the advantages of MCs as a product that proved its worth for the producer. Recent investigations, ranging from field studies to laboratory experiments provided some insights into the different potential intra- and extracellular functions of MCs.

First, MCs are known as cyanobacterial secondary metabolites; however, the evidence obtained by current studies indicated they are produced continuously from the early logarithmic phase to the late stationary phase, making this definition unlikely (Orr and Jones 1998, Lyck 2004, Gantar *et al.* 2008, Tonietto *et al.* 2012). Moreover, current studies clarified the probable metabolic roles of MCs as an essential cellular compound (Gantar *et al.* 2008). Therefore, there might be a close connection between toxin production and the primary metabolism of toxigenic cyanobacteria (Lyck 2004, Zilliges *et al.* 2011, Holland and Kinnear 2013).

Secondly, MCs are known as endotoxins, but they can be released into the surrounding environments at different growth stages or under diverse environmental conditions (Hotto 2007). Moreover, the discovery of an ABC transporter encoded by a part of the *mcy* gene cassette strengthens the theory of possible MC export (Dittmann *et al.* 2001). However, the extracellular

toxin fraction constitutes about 10% of the total produced MCs; it's possible physiological and / or ecological importance especially in dense blooms might be considerable (Dittmann and Börner 2005).

On the other hand, the necessity of MCs in the central metabolic pathway is still doubtable due to the existence of non-toxic strains that contain the *mcy* gene cluster but do not produce any detectable MCs (Christiansen *et al.* 2008). However, proteomic studies revealed that different quantity and forms of the same proteins were expressed in toxic and non-toxic strains (Tonietto *et al.* 2012). Moreover, in cyanobacterial blooms toxic and non-toxic strains co-existed. How can we explain this occurrence? Perhaps non-MC-producing strains exploit the available extracellular toxins produced by the toxic genotypes in blooms, or they might produce other metabolites with the similar chemical structure but which are not toxic such as cyanopeptolines or microginins (Namikoshi and Rinehart 1996, Leflaive and Ten-Hage 2007).

Cyanobacterial blooms contain a mixture of toxic and non-toxic strains in various proportions. The population dynamics of cyanobacterial blooms also change with time during bloom formation. Under favorable growth conditions, non-toxic strain might outcompete toxic populations. In contrast, under various stress conditions induced by biotic and abiotic factors such as nutritional limitations, which often accompany increased blooms density, the toxic strains can survive longer by virtue of their toxicity (Kardinaal *et al.* 2007, Renaud *et al.* 2011, Briand *et al.* 2012). Indeed, the strains which are equipped with the stress adaptation systems can dominate the competition with others in natural ecosystems.

Stress-induced conditions such as high light intensity, iron deficiency, and exposure to hydrogen peroxide promote MCs attachment to certain proteins (Zilliges *et al.* 2011). Covalent binding of MCs to proteins that are involved in carbon and nitrogen metabolism might increase the stability of these proteins against oxidative stress-induced damage and redox changes. As a result, the proteins maintain their natural conformation or activity under various stresses. Moreover, containing a higher quantity of proteins involved in cellular metabolism by toxigenic strains might lend the advantage of providing a larger cellular metabolic reservoir to endure longer under limited nutritional conditions or to produce more ATP which makes the cost of toxin production meaningful (Tonietto *et al.* 2012). Under oxidative stress, more MCs are released to the surrounding environment and therefore might play a role as a signal molecule to synchronize the cell responses to the stress-inducing agents. Increased intracellular MC concentration in response to oxidative

stress conditions such as iron limitation support the hypothesis of MCs involvement in protection against oxidative stress, while the enhancement in extracellular MCs suggests the possible role of MCs as a signal molecule to make a better environmental adaptation (Yeung *et al.* 2016). Additionally, *in vitro* MC-metals complex formation, besides MC localization in polyphosphate bodies, supports the possibility of MCs involvement in metal detoxification or storage within the producer organism.

On the other hand, some current findings highlight the involvement of MCs in central metabolism. Differences in the proteins related to the carbon and nitrogen metabolism between MC-producing and non-toxic strains provides new insights into the possibility of MCs involvement in the cellular processes. The proximity of MCs to thylakoid membranes and carboxysomes, aside from the evidence of regulation of both toxicity and photosynthesis efficiency by light, suggests a possible role of MCs in photosynthesis and carbon fixation. Identification of binding sites of the nitrogen regulator NtcA in the *mcy* gene cluster promoter, as well as the dependency of MC synthesis on nitrogen supplies and the carbon/nitrogen status, suggest a possible link between toxicity and primary cellular metabolism.

Light-regulated proteins similar to the proteins involved in signal transduction and the regulatory effect of light on MC synthesis propose that *Microcystis* might have a light sensing mechanism with the features of a quorum sensing system in which light intensity can be considered as a controlling agent (Dittmann *et al.* 2001). Moreover, finding of a Fur binding sequence in the promoter region of the *mcy* gene cluster and increased iron uptake under high light reinforces the possible role of MCs in iron acquisition regulated through a light signaling cascade.

From an ecological point of view, the importance of MCs has been more elucidated by studies of interactions between *Microcystis* and other aquatic organisms. MC's role in the maintenance of bloom formation and as a feeding deterrent offers defense mechanisms against grazers linked to MC production (Gan *et al.* 2012). Furthermore, toxin related interspecies interactions, allelopathy, resulted in the suppression of growth and photosynthesis together with induction of oxidative stress in other aquatic species, especially the members of the phytoplankton community This may improve success leading to cyanobacterial dominance, a critical function of MCs for the producer to dominate over other phytoplankton community members. MCs play a role in the benthic survival of *Microcystis* vegetative cells and as a signal molecule in the regulation of *Microcystis* recruitment, an important process for the annual succession of *Microcystis* blooms.

However, conflicting results in different studies, possibly due to the lack of standardized experimental designs and analysis using different culturing methods and growth conditions, have limited a generalized definition of the role of MCs and applying it to MC-producing species other than *M. aeruginosa* (Kardinaal *et al.* 2007, Neilan *et al.* 2013). For instance, studies of the effects of various light intensities on MC production showed controversial results. Some studies indicated that MC production was elevated at high light intensity while the other studies revealed that it was increased under low light intensity (Van der Westhuizen and Eloff 1985, Sivonen 1990, Utkilen and Gjølme 1992, Rapala *et al.* 1997, Kaebernick *et al.* 2000). Strain-specific toxin production rate has further hindered the comparison of studies and interpretation of tested parameters. For instance, the presence of some proteins such as MrpC are highly strain specific and may be associated with distinct colony types of *Microcystis* (Zilliges *et al.* 2008, Gan *et al.* 2012). On the other hand, controlled laboratory conditions might be different from the natural conditions where a variety of complex biotic and abiotic factors influence cyanobacterial proliferation and toxin production. It is further necessary to realize that laboratory experiments do not provide a holistic view of the function of MCs as *Microcystis* behaves differently under laboratory conditions compared to in nature as is evident with colony formation in nature versus unicellular growth when cultured (Zhang *et al.* 2007, Sun *et al.* 2015). In addition to the morphological shifts, more changes have been reported in *Microcystis* strains isolated from the field and cultured in the laboratory such as loss of MC production ability or changes in the toxicity and MCs variants (Cuvin-Aralar *et al.* 2002, Schatz *et al.* 2005). On the other hand, previously known non-toxic strains might retain the genes for MCs synthesis (Rantala *et al.* 2004). These changes have made it difficult to obtain a clear understanding of what happens in nature. For instance, the different defense mechanism against grazers has been reported for the unicellular laboratory cultures and colonial field samples. For the unicellular cultures, toxicity, and in the case of the field samples, large colony formation, have been explained as defense mechanisms.

Consequently, despite the vast amount of research on MCs, a general function has not been elucidated, but it is possible that MC can have multiple functions. There are still many raised questions that need to be answered. Further studies are required to clarify the natural role(s) of MCs.

In the end, evidence suggests that global warming and eutrophication will increase the occurrence of cyanobacterial blooms with a shift toward more toxic populations in the future. Therefore, deeper studies need to get more insights into the success of *Microcystis* in making

blooms worldwide. Improvement of the current knowledge concerning the ecological or physiological significance of MCs and the conditions influencing MC production might open the way for the control of harmful cyanobacterial blooms. For instance, allelochemicals produced by co-occurring organisms might be useable as eco-friendly biocontrol agents of *Microcystis* blooms. Therefore, further understanding of allelopathy can aid in biological bloom control in the future.

Acknowledgments

This study was sponsored by Elsa-Neumann-Scholarship (formally known as NaFöG) from the state of Berlin.

Disclosure of interest

The authors report no conflicts of interest.

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Table 1. MCs possible intracellular functions

Role	Evidences	References
Photosynthesis and Light adaptation	Regulation of MC production rate and transcript levels of *mcy*D by the light intensity	(Utkilen and Gjølme 1992, Wiedner *et al.* 2003, Renaud *et al.* 2011)
	Higher MC production during the light phase of light – dark cycle	(do Carmo Bittencourt-Oliveira *et al.* 2005)
	Direct relation of MCs content to chlorophyll a quota	(Van der Westhuizen and Eloff 1985, Long *et al.* 2001, Debolis and Juneau 2010)
	Light limitation • Lower photosynthetic pigments in MC-deficient mutant • Higher ratio of phycocyanine to Chlorophyll a	(Hesse *et al.* 2001)
	Higher photosynthesis rate in *Microcystis* PCC 7806	(Meissner *et al.* 2015)
	Localization of MCs in thylakoids membrane	(Shi *et al.* 1995, Young *et al.* 2005, 2008)
	Different expression of two NADPH-dependent reductases, phycobiliproteins and RuBisCo in *Microcystis* wild type and *mcy*B$^-$ mutant	(Zilliges *et al.* 2011)
	High light irradiation	
	• Dominance of toxic strain in a mixed culture of toxic-non-toxic strains	(Renaud *et al.* 2011)
	• Higher percentage of MCs close to the thylakoids membrane	(Gerbersdorf 2006)

	• Enhancement in MC attachment to certain proteins	(Zilliges *et al.* 2011)
	• Faster accumulation of carbon reserves • Greater contents of high molecular weight carbohydrate in the wild type of *M. aeruginosa*	(Meissner *et al.* 2015)
Environmental adaptations	Temperature	
	Enhanced MCs by increased temperature to the optimal growth temperature and decreased MCs quantity at exceeded temperatures	(Van der Westhuizen and Eloff 1985)
	Increased temperature	
	• Stimulation of *Microcystis* blooms formation in summer	(Liu *et al.* 2011, O'Neil *et al.* 2012)
	• Greater proportion of toxic population in the lakes	(Dziallas and Grossart 2011)
	• Higher frequency of *Microcystis* toxic blooms	(Davis *et al.* 2009)
	• Higher ratio of toxic vs. non-toxic strains of *M. aeruginosa* • Higher *mcy*D transcript levels • Greater proportion of MC-LR to MC-YR in a mixed culture with heterotrophic bacteria	(Dziallas and Grossart 2011)
	pH higher or lower than the pH 9 • Increased toxicity	(Van der Westhuizen and Eloff 1983)
	Toxic *Microcystis* sp. (KLL strain MG-K) outcompete the non-toxic laboratory emerged successor (strain MG-J)	(Schatz *et al.* 2005)
	Dissolved inorganic carbon (DIC)	
	Low and high concentrations of DIC • Greater growth at lower DIC and no change in the chlorophyll content of *M. aeroginosa*	(Zhang *et al.* 2012)

	• Lower photosynthesis efficiency and chlorophyll content of *Chlamydomonas microsphaera*	
	Increased DIC (Lake Chaohu, China) • Lower abundance of toxic *Microcystis* and MCs quota	(Yu *et al.* 2014)
	Decreased DIC • Increased MCs production • Greater chlorophyll a content of *M. aeruginosa* PCC 7806 wild type	(Jähnichen *et al.* 2007)
	• Growth inhibition of non-toxic laboratory emerged successor by the toxic *Microcystis* sp • Survival of toxic strain and lysis of non-toxic spontaneous mutant during the bloom season	(Schatz *et al.* 2005)
Protection against oxidative stress	Exposure to hydrogen peroxide • Longer survival of toxigenic *Microcystis* • Lesser decrease in its chlorophyll a contents	(Dziallas and Grossart 2011)
	Increased temperature • Higher increase in MCs content and reactive oxygen species	(Dziallas and Grossart 2011)
	Nitrogen and phosphorus limitation • Increased MC production and *mcy*D transcript levels	(Pimentel and Giani 2014)
	Iron depletion • Increased intracellular MCs in *M. aeruginosa* PCC 7806 • Reduction in the cell size and chlorophyll a content	(Yeung *et al.* 2016)
	High light irradiation	(Meissner *et al.* 2015)

	• Increased stress markers in *M. aeruginosa mcy*B$^-$ mutant	
	Oxidative stress • Enhancement in *mcy* gene cluster transcripts	(Straub *et al.* 2011)
	Different expression of proteins phycobiliproteins, CpcB and ApcA, RuBisCo, glutathione reductase and some hypothetical proteins in the wild type and mutant strain	(Zilliges *et al.* 2011)
	High light, iron deficiency and exposure to hydrogen peroxide • Stimulation of MC-protein binding in *M. aeruginosa* PCC 7806 wild type	(Zilliges *et al.* 2011)
	Iron depletion • Greater tendency of MC to the binding sites of thioredoxin-regulated proteins	(Alexova *et al.* 2016)
	High light (51800 lm m^{-2}) • More stability of the large subunit of RuBisCo in the wild type	(Zilliges *et al.* 2011)
	High light • Decrease in oxygenase function of RuBisCo, Protection against photorespiration	(Gerbersdorf 2006)
	C-limitation • Higher intracellular and extracellular MCs quota • Higher concentration of chlorophyll a	(Jähnichen *et al.* 2007)
	MC binding to metals such as zinc and cadmium	(Dziallas and Grossart 2011)
	MC as additional radical scavengers	(Zilliges *et al.* 2011)
Nutrient metabolism and	Dependency of MC production on the nitrogen concentration	(Harke and Gobler 2013, Holland and Kinnear 2013)

storage	Binding site of NtcA to the promoter region of *mcy* gene cluster	(Kuniyoshi *et al.* 2011)
	Nitrogen starvation • Increased *ntc*A transcripts • Decreased *mcy* gene cluster expression	(Kuniyoshi *et al.* 2011, Harke and Gobler 2013, Pimentel and Giani 2014)
	2-oxoglutarates addition • Increased binding of NtcA to the *mcy*A promoter • Decreased *mcy* gene expression.	(Kuniyoshi *et al.* 2011)
	Toxic and non-toxic strains are different in • The isoforms of the same proteins • Quantities of proteins involved in carbon-nitrogen metabolism	(Tonietto *et al.* 2012)
	Nitrogen depletion • Greater accumulation of proteins involved in carbon and nitrogen metabolism in non-toxic *M. aeruginosa* PCC 7005 and *mcy*H$^-$ mutant	(Alexova *et al.* 2016)
	Localization of MCs within carboxysomes	(Gerbersdorf 2006)
	MCs in the vicinity of polyphosphate inclusion bodies	(Gerbersdorf 2006)
	C-limitation	
	• Better adaptation of *M. aeruginosa* PCC 7806 wild type • Higher intracellular and extracellular MCs quota • Higher concentration of chlorophyll a	(Jähnichen *et al.* 2007)
	• Inhibition of oxygenase function of RuBisCo	(Jähnichen *et al.* 2001, 2007)
	Higher phosphorus concentration • Greater ratio of toxic to the non-toxic population	(Davis *et al.* 2009, Yu *et al.* 2014)

	Phosphorus limitation • Increased MCs content • Higher ratio of MC-LR to MC-RR • Lower carbon fixation rate	(Oh *et al.* 2000)

Table 2. MCs possible extracellular functions

Role	Evidences	References
Quorum sensing	Detection of extracellular MCs	(Hotto 2007)
	Homology of McyH to the ABC transporter	(Pearson *et al.* 2004)
	No detection of MCs in the Δ*mcy*H mutant of *M. aeruginosa*	(Pearson *et al.* 2004, Kaplan *et al.* 2012)
	Increased cell density	
	• Increased MC production rate	(Wood *et al.* 2012)
	• Higher *mcy*E transcripts • Increased total and extracellular MC quota	(Wood *et al.* 2011, 2012)
	Exposure to pure MC-LR or crude extract • Increased *mcy*B transcript levels	(Schatz *et al.* 2007)
	Iron limitation • Increased extracellular MCs	(Yeung *et al.* 2016)
	Similarity between the sequence of the *mcy* gene cluster of *M. aeruginosa* and quorum sensing genes of *R. leguminosarum*	(Dittmann *et al.* 2001)
	Similarity between light regulated proteins MrpA & B of *M. aeruginosa* PCC7806 and RhiA & B, signal mediator proteins of *R. leguminosarum*	(Dittmann *et al.* 2001)
	• Strong expression of MrpA in the wild type • No detection of MrpA in a *mcy*B$^-$ mutant. • Enhancement in the transcription levels of MrpA & B proteins in response to MC addition	(Dittmann *et al.* 2001)
Benthic survival and recruitment	Survival of *Microcystis* cells at the surface and depth of sediments	(Ihle *et al.* 2005, Latour *et al.* 2007, Misson *et al.* 2012a, 2012b,

		Catherine *et al.* 2013, Torres and Adámek 2013)
	MCs detection in benthic sediments	(Latour *et al.* 2007)
	Preservation of MCs synthesize after several years of sedimentations (lake Grangent, France)	(Misson *et al.* 2012a)
	Decrease in cellular MCs quota by increase in the age of sediments	(Misson *et al.* 2012a, 2012b)
	preferred recruitment of toxic *Microcystis* to non-toxic subpopulation (Lake Quitzdorf, Germany)	(Schöne *et al.* 2010)
	Greater recruitment rate of smaller colonies (> 160 µm) with higher MCs content (Lake Villerest (France))	(Misson *et al.* 2011)
	Annual decrease in *Microcysis* population in sediments during spring	(Ihle *et al.* 2005)
	MC-LR addition • Greater decrease in recruitment of toxic *Microcystis*	(Misson *et al.* 2012a)
Colony formation	Exposure to *Ochromonas* spp. • Induction of colony formation in *M. aeruginosa* PCC 7806	(Yang *et al.* 2009, Yang and Kong 2012)
	MC-RR addition • Increased extracellular polysaccharides and larger colony formation	(Gan *et al.* 2012)
	Larger colonies (>100 mm) (Lake Wannsee Germany) contained • Greater ratio of toxic to non-toxic genotypes	(Kurmayer *et al.* 2003)
	• Covalently binding of MCs to lectin microvirin protein • Lower MC production in microvirin-deficient mutant	(Kehr *et al.* 2006)

	• Different kinds of microvirin detected in non-toxic strains	
	MC-deficient mutants • Higher accumulation of MrpC • Greater aggregation tendency	(Kehr *et al.* 2006, Zilliges *et al.* 2008)
Defense against grazers	Exposure to grazers • Colony formation, lower long chain saturated fatty acids production and increased toxicity in *Microcystis*	(Müller-Navarra *et al.* 2000, Jang *et al.* 2003, Watson 2003, Yang and Kong 2012)
	Exposure to zooplanktons *M. macrocopa*, *D. magna* and *D. Pulex* • Increase in MC production depended on the growth stage, population densities and concentrations of cellular exudates	(Jang *et al.* 2003)
	MC synthesis triggered by infochemicals released from herbivorous zooplankton	(Jang *et al.* 2007a, Jang *et al.* 2008)
	Repaid death and decrease in the mobility of *D. galeata* fed with the *M. aeruginosa* PCC7806 wild type	(Rohrlack *et al.* 1999b)
	Negative correlation between distribution and density of *Daphnia* to *Microcystis* blooms	(Reichwaldt *et al.* 2013)
	Exposure to phytoplanktivorous and omnivorous fish • MCs increased in response to released infochemcals	(Jang *et al.* 2004, Ha *et al.* 2009)
Iron uptake or transfer	Dependency of MC production rate to iron concentration	(Zakhia *et al.* 2008)
	Higher toxicity under low iron concentration	(Lukač and Aegerter 1993)

	Severe and long-term iron limitations • Longer survival of MC-producing strain with a greater iron uptake and higher MCs production	(Martin-Luna *et al.* 2006a, Alexova *et al.* 2011)
	Iron depletion • Increased MCs • Greater accumulation of phycobilisome proteins and FutA in toxic *M. aeruginosa* PCC 7806	(Alexova *et al.* 2016)
	Low affinity of MCs to ferric iron	(Klein *et al.* 2013)
	Binding site of Fur in the promoter region of *mcy* gene cassette	(Martin-Luna *et al.* 2006b, Kaplan *et al.* 2012)
	Iron depletion • Higher expression of Fur • Increased MC production rate	(Martin-Luna *et al.* 2006a, 2006b)
	High light • Increase in the iron uptake	(Kaebernick and Neilan 2001)
Allelopathic interactions	Exposure to MC-LR	
	• Growth inhibition of various algal species	(Kaebernick and Neilan 2001, Singh *et al.* 2001, Babica *et al.* 2006, Leão *et al.* 2009)
	• Growth inhibition • Photosynthesis inhibition in *Ceratophyllum demersum* and *Myriophyllum spicatum*	(Pflugmacher 2002)
	Increase in antioxidative enzymes activity in *Ceratophyllum demersum*	(Pflugmacher 2004)
	Exposure of alfalfa (*Medicago sativa*) seedlings to MC-LR	(Pflugmacher *et al.* 2006)

	• Inhibition Germination and root development • Oxidative stress induction	
	• Promotion of oxidative stress in *Cyprinus carpio L.*	(Jiang *et al.* 2013)
	Cell-free filtrate of *Microcystis* sp. (KLL strain MB) • Growth and photosynthesis inhibition of dinoflagellate *Peridinium gatunense*	(Sukenik *et al.* 2002)
	Exposure of *S. armatus* and *C. demersum* to the crude extract of a cyanobacterial bloom • Oxidative stress induction • Photosynthesis inhibition	(Pietsch *et al.* 2001)
	Direct exposure of *Lemna japonica Landolt* to *M. aeruginosa* • MCs increase and growth inhibition of *M. aeruginosa* • Growth inhibition of *L. japonica*	(Jang *et al.* 2007b)
	Co - cultivation of *M. aeruginosa* and *Chlorella* sp. • Growth stimulation and then inhibition by increase in the initial density of *Microcystis*	(Hong *et al.* 2010)
	Co - cultivation of *M. aeruginosa* CPCC 299 and *P. agardhii* NIVA-CYA 126 • Decline in the biomass, *mcyE* gene copies and McyE transcripts of both species	(Ngwa *et al.* 2014)
	Exposure to hydrogen peroxide or increased temperature • Greater MCs content in xenic cultures of *M. aeruginosa* PCC 7806 containing heterotrophic bacteria	(Dziallas and Grossart 2011)
	Indirect exposure to phytoplanktivorous and omnivorous fish	(Jang *et al.* 2004, Ha *et al.* 2009)

	• Greater MC production in response to the released infochemcals	

Figures

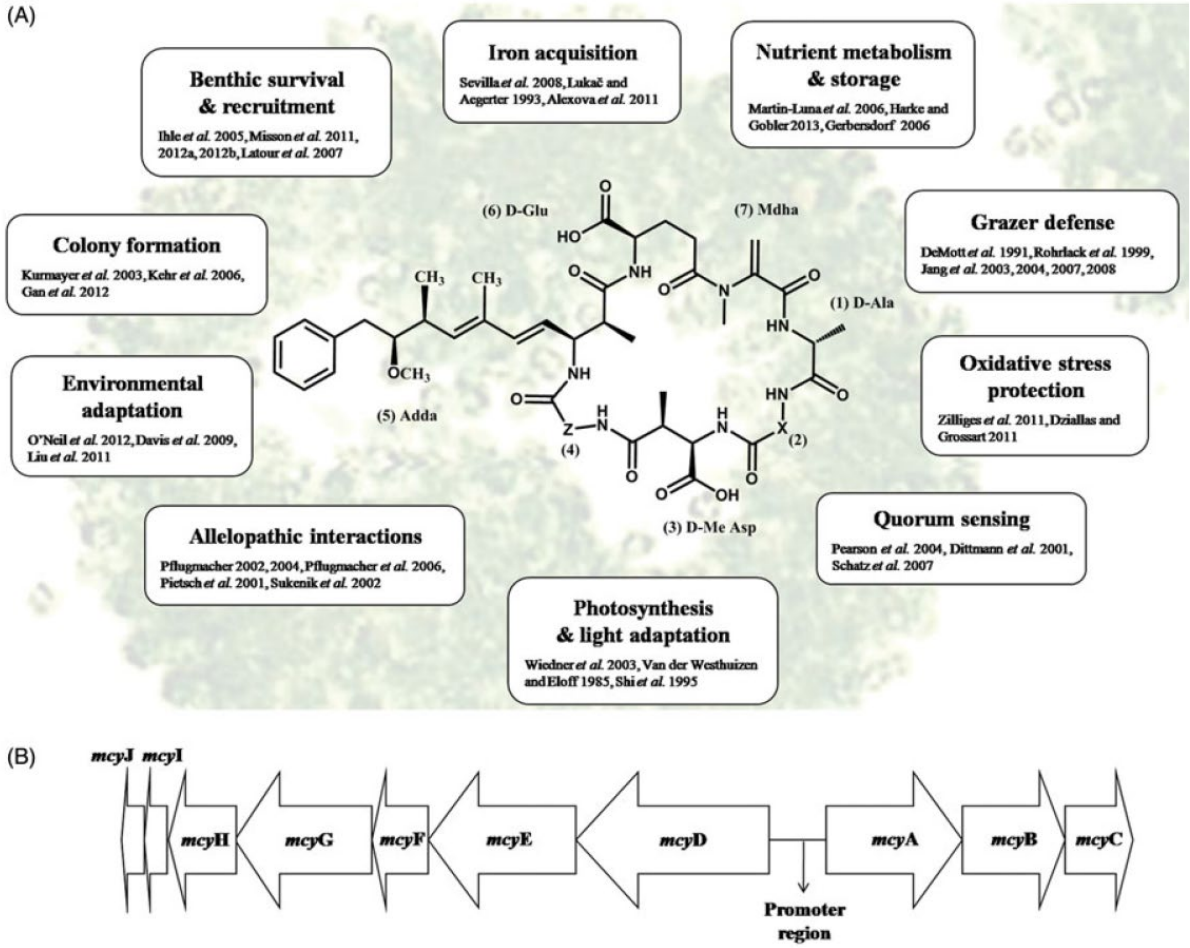

Figure 1. (A) The chemical structure of MCs and their proposed intracellular and extracellular functions and (B) MC synthesis gene cluster (*mcy*)