Contribuţii Botanice, XLI, (1), **2006**
Grădina Botanică "Alexandru Borza"
Cluj-Napoca

REVIEW: MICROCYSTINS: POWERFUL CYANOBACTERIAL TOXINS

Daniela Alina BOARU[1], Kristin SCHIRMER[2], Corneliu TARBA[1], Nicolae DRAGOŞ[1]
[1.] Universitatea "Babeş-Bolyai", Facultatea de Biologie şi Geologie, str. M. Kogălniceanu, nr. 1,
RO-400084 Cluj-Napoca
[2.] Department of Cell Toxicology, UFZ Centre for Environmental Research Leipzig-Halle
in the Helmholtz Association, **D-04318 Leipzig**
e-mail: ndragos@hasdeu.ubbcluj.ro

Abstract: Toxins producing species of cyanobacteria have been detected worldwide in fresh and marine waters. Tthe most commonly cyanotoxins found in the aquatic medium are the hepatotoxic microcystins. These toxins are responsible for numerous cases of poisoning and death of animals and present a potential hazard to human health. The water eutrophication can enhance the toxic cyanobacteria growth hence increasing the risk of exposure to microcystins. The present paper aims at providing a short overview of cyanobacteria forming water-bloom and their ability to synthesize cyanotoxins, focusing on MCYSTs biosynthesis, occurrence and their in vitro and in vivo toxic effects. Microcystins toxic effects are largely due to the inbition of protein phosphatase and induction of oxidative stress. Consequences of exposure to high levels of microcystins include structural damage and apoptosis, whereas the exposure for long periods of time to lower toxin concentrations can lead to liver tumours.

Keywords: cyanobacteria, microcystins, toxic effects, apoptosis, tumour promotion, *in vitro*, *in vivo*.

Introduction

Economic development during the last centuries has led to a severe degradation of the environment, with water being a heavily affected resource. Residential, agricultural and industrial activities are linked to production and release of huge amounts of chemicals. These chemicals enter the water by direct discharge as waste, by runoff from agricultural land or by disposition from air. Their presence in the aqueous environment changes the regular chemical composition, which can, for example, be reflected in an altered taste or smell. Therefore, it is not surprising that the man-made input of chemicals into the water, which is generally referred to as water pollution, may also impact on the composition and/or health of the organisms living in this environment.

The nature and sources of water pollution can be manifold. However, a major cause of pollution is the high input of nutrients such as nitrates and phosphates. The main source of the nitrate pollution is runoff of fertilizers from agricultural land, whereas most phosphorus pollution comes from industry (e.g. detergents based on phosphorus). Enrichment of water bodies with nitrates and phosphates is also known as eutrophication. This phenomenon in association with other factors, such as proper temperature and sunlight, can lead to over-stimulation of macrophyte, algal and cyanobacterial growth. Among these, cyanobacteria are a prokaryote photosynthetic group of microorganisms that cause significant environmental and human health concern.

Accelerated growth of cyanobacteria leads to the occurence of massive blooms, which can accumulate in large mats. These mats disturb the balance of organisms present in the water by using up the dissolved oxygen and by reducing light penetration into deeper waters. Importantly, some of cyanobacteria have the ability to release toxic metabolites, called cyanotoxins [24].

The cyanotoxins include a wide spectrum of chemically diverse substances that act as potent neurotoxins (e.g. anatoxins), dermatotoxins (e.g. lyngbyatoxins) and hepatotoxins (e.g. microcystins). Exposure to these toxins can cause severe illness in animals and humans and in some cases cause death. The earliest record of acute cyanotoxin poisoning of domestic animals

was reported in *Nature* journal by G. Francis [73]. Cyanobacterial-based poisonings of farm animals, wildlife and humans involving cyanobacterial water-blooms are now frequently reported from large areas of the world [25, 81]. Observations of human deaths caused by acute exposure to cyanotoxins are limited to a single reported case so far. In 1996, several cases of liver failure and human death occurred in a Brazilian dialysis clinic, being caused by the exposure of the patients to cyanotoxin-contaminated water used for dialysis (Caruaru syndrome) [29, 157]. Cyanotoxins do not have to be present in high concentration in the consumed water in order to have a harmful effect on humans. Chronic exposure to low levels of microcystins through drinking water has been linked to the development of liver cancer in people living in some areas in China [216].

It is very probable that the occurrence of cyanobacteria in the aquatic environment, including associated toxin production, will continue to increase as a result of human activity. This is one reason why cyanobacteria and related toxins have recently become the object of an increasing number of government-sponsored research projects. It is of further interest to use these toxins as potential drugs, e.g. for anti-cancer treatment [196, 197]. Among the environmentally relevant cyanotoxins, the hepatotoxic microcystins (MCYSTs) are the most commonly found, which might be explained by their relative stability in the aquatic environment [105]. Microcystins are also the cyanobacterial toxins most frequently associated with animal and human poisonings. The present paper aims at providing a short overview of cyanobacteria forming water-bloom and their ability to synthesize cyanotoxins, focusing on MCYSTs biosynthesis, occurrence and their *in vitro* and *in vivo* toxic effects.

1. What cyanobacteria are?

Cyanobacteria are an ancient group of organisms comprising more than 150 genera and 2000 species [34]. Their ecological importance lies in their role in the global carbon, oxygen and nitrogen cycles. Fossil studies showed that cyanobacteria have been present on the Earth for about 3.3-3.5 billion years [27, 28]. Therefore, they were among the pioneer organisms of early earth and had an important role in the oxygen evolving in the ancient atmosphere [18, 174].

One characteristic feature of cyanobacteria is their bluish colour, the cellular phycocyanin (a blue phycobilin pigment) and chlorophyll *a* being responsible of their pigmentation. The colour of blooms and several other generic similarities between cyanobacteria and eukaryotic microalgae led to the early name “blue-green algae” for these microorganisms. However, their cellular structure and functions are of bacterial type, which explains their relatively recent designation as cyanobacteria. Being equipped with a photosynthetic apparatus similar to that of the plastids, cyanobacteria have ability, just as algae, of performing oxygenic photosynthesis. Yet, like prokaryotes, they do not possess a nucleus [89] nor are they equipped with organelles. They have a wall structure based on a peptidoglycan layer and contain 70S rather than 80S ribosomes [19, 67].

Cyanobacteria are also known by their considerable morphological diversity, rare for the prokaryote world [17, 18]. The basic morphology comprises unicellular, colonial and multicellular filamentous forms. Cyanobacteria are able to establish symbiotic associations with a large variety of organisms, such as gymnosperms, angiosperms, protozoa, bryophytes, pteridophytes, and fungi [124, 159]. The lichens, where the phycobiont is represented either by cyanobacteria or by green algae, are a classical example. There is a general acceptance in connecting the cyanobacteria with the endosymbiotic origin of plastids found in algae and higher plants [52]

Cyanobacteria are the first to colonize bare areas and inhabit very diverse environments, including arctic lakes, thermal waters (with temperatures exceeding 70-73ºC), desert sands, rocks, volcanic ash [34] and, of course, all surface waters, from tropical to temperate zones. In continental waters, cyanobacteria are quite abundant, showing a high ecological plasticity, benthic and planktonic species are frequently present together in water bodies. The planktonic species

posses gas vesicles, which enable them to adjust their position in the water column [208], to float on water surfaces and, under appropriate conditions, to develop blooms which can accumulate as scum. The scum formation increases many times the detrimental aspects of a cyanobacterial bloom because of a generalized cell lysis followed by release and increased aqueous concentrations of cyanotoxins at harmful levels.

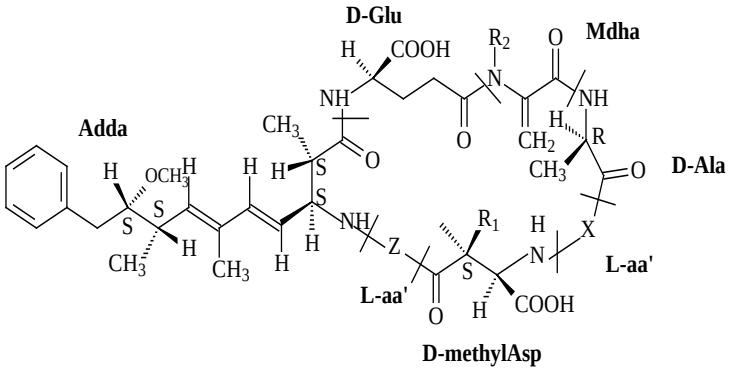
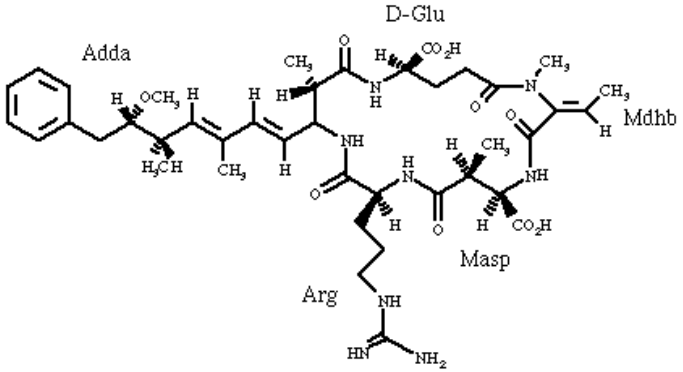
Studies on water blooms of planktonic and mass developments of benthic cyanobacteria in different countries revealed that about 25% to 90% of the blooms are toxic [5, 23, 37, 179]. The cyanotoxins are considered to be present mainly within the cyanobacterial cell and readily available in high concentration in water after cellular senescence and lysis [150, 180]. Some researchers [151] have reported a release of MCYSTs out of young growing cyanobacterial cells. Recent identification of a putative ABC transporter gene (*mcy*H), a part of the *mcy* gene cluster involved in microcystin production, may suggest the existence of an active microcystin transport system. The possibility of such active release, however, requires further investigation.

2. Cyanotoxins

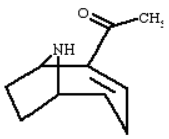
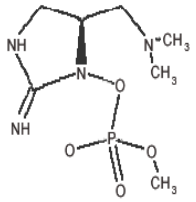
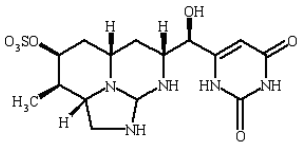
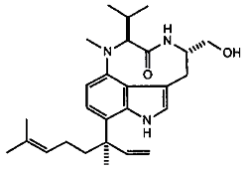
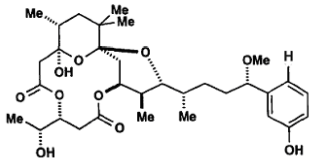
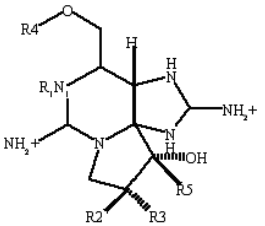
Based on their chemical structure, cyanotoxins have been separated in three groups: cyclic peptides, alkaloids and lipopolysaccharides (LPS). Table 1 summarizes the general features of these groups. The cyclic peptides comprise the microcystins (MCYSTs) and the nodularins, named according to their producers.

Both compound classes present many structural variants. All variants are powerful hepatotoxins, which target the liver and act as inhibitors for protein phosphatases [57]. Whereas MCYSTs are cyclic heptapeptides containing two protein amino acids and five non-protein amino acids, the nodularins have only five amino acids in their peptide ring. The overall structure of microcystins and nodularins is similar, a fact that may explain their identical hepatotoxic action (Table 1). Furthermore, the gene cluster involved in their biosynthesis suggests a common evolution of these toxins, indicating that nodularin is a variant of MCYSTs class (discussed below) [161]. The alkaloids anatoxin-*a*, anatoxin-*a* (S), and saxitoxins (paralytic shellfish poisons) are neurotoxins that act by blocking synaptic transmission [3, 5, 98, 205]. In general, the first two act at cholinergic synapses. Anatoxin-*a* mimics acetylcholine and binds irreversibly to its nicotinic receptor at neuromuscular junctions, and also throughout the central nervous system. Acetylcholinesterase is not able to displace the bound cyanotoxin. As a result, overstimulation at the level of synaptic junction occurs. If the chest muscles responsible for respiration are affected, convulsions appears due to a lack of oxygen supply to the brain. Suffocation is the final result and occurs few minutes after toxin ingestion. Anatoxin-*a* (S), in turn, binds to and inactivates acetylcholinesterase, the enzyme responsible for acetylcholine degradation. Anatoxin-*a* (S) triggers reactions such as excessive salivation, urination and defecation and, like anatoxin-*a*, causes severe respiratory problems. Saxitoxin binds to the voltage-dependent sodium channels in brain, peripheral nerves and skeletal muscles and blocks the transmission of the nerve impulse. The symptoms are paralysis followed by respiratory failure. While anatoxins seem to be unique for cyanobacteria, the saxitoxins are also produced by various dinoflagellates [180]. The other cyanotoxins of alkaloidic nature are cylindrospermopsins, lyngbyatoxins and aplysiatoxins. Cylindrospermopsins elicit hepatotoxic effects, whereas lyngbyatoxins and aplysiatoxins are dermatotoxins. The latter two are generated mainly by tropical and subtropical marine benthic cyanobacteria. The lipopolysaccharides (LPS) are cell wall components of all Gram-negative bacteria, including cyanobacteria. Cyanobacterial LPS cause irritation and allergic reactions that may potentially affect any exposed tissue. Keleti and Sykora [108] have shown that cyanobacterial LPS generally have a lower biological activity than LPS from other Gram-negative bacteria.

Table 1: General informations about the cyanotoxins including their target organs and the cyanobacterial genera known to produce them.

	Cyclic peptides	
Cyanobacteria genera	***Microcystis, Anabaena, Nostoc, Oscillatoria, Anabaenopsis, Hapalosiphon***	***Nodularia***
Toxin group	**Microcystins**	**Nodularins**
		
Primary target	**Liver**	**Liver**

Alkaloids

Cyanobacteria genera	***Anabaena, Aphanizomenon, Oscillatoria, Thrichodesmium***	***Anabaena***	***Aphanizomenon, Cylindrospermopsis, Umezakia***
Toxin group	**Anatoxin-a**	**Anatoxin-a (S)**	**Cylindrospermopsin**
			
Primary target	**Nerve synapse**	**Nerve synapse**	**Liver**
Cyanobacteria genera	***Lyngbya***	***Oscillatoria***	***Lyngbya,Anabaena, Aphanizomenon, Cylindrospermopsis***
	Lyngbyatoxin-a	**Debromoaplysiatoxin**	**Saxitoxins**
			
Primary target	**Skin, Gastro- intestinal tract**	**Skin**	**Nerve axon**

Lipopolysaccharides

Cyanobacteria genera	***All***
Toxin group	**LPS**
Primary target	**Potential irritant, affects any exposed tissue**

Thus, among all of the cyanobacterial toxins, the hepatotoxic MCYSTs are so far considered the most hazardous. This is partly reflected by the attention they have been gaining recently by the scientific community. For example, a quick PubMed search reveals 1028 publications on MCYSTs (since 1981) compared to 777 for anatoxins and only 72 for cylindrospermopsins. One of the MCYSTs variants, MCYST-LR, is also the only cyanobacterial toxin for which a regulatory level of acceptance exists. In 1998, the World Health Organization established a provisional guideline level of 1 μg/L for this MCYST in drinking water [1].

3. The microcystins (MCYSTs)

The more than 80 MCYSTs congeners known thus far are produced by a wide range of cyanobacteria, including species of *Microcystis*, *Anabaena*, *Oscillatoria* (*Planktothrix*), *Nostoc*, *Hapalosiphon* and *Anabaenopsis* genera [89]. Within the species, there can be both toxic and non-toxic strains. Inside the *Microcystis* genus, *M. aeruginosa* and *M. viridis* species are commonly associated with cyanobacterial blooms and MCYSTs production [107, 203, 212]. The production of MCYSTs by cyanobacteria is a very complex process modulated by a combination of genetic and ecological factors ([180]; see also section 3.1. and 3.2.). A cyanobacterial bloom can be dominated by a single species or by a variety of species that may or may not differ in their ability to produce MCYSTs. Furthermore, a single cyanobacterial species has ability of producing more than one MCYST variant as shown e.g. for *M. aeruginosa*-dominated blooms [53, 193].

The different MCYSTs variants described so far have been isolated directly from bloom samples or from cyanobacterial strains cultivated in the laboratory. The congeners are collectively referred to as MCYSTs because the first hepatotoxic compound from this class was isolated from *M. aeruginosa* strain NRC-1 (SS-17) [8, 111] and the first MCYST chemical structure described was that of MCYST-LR [165]. Structurally, MCYSTs are monocyclic heptapeptides with low molecular weight ranging between 909-1115 Da and containing seven amino acids. Two of them are variable L-amino acids (L-AA), and two are special D-amino acids (D-AA). The general structure of MCYSTs is: D-alanine at position 1, the two variable L-AAs at position 2 and 4, γ-linked D-glutamic acid at position 6, β-linked D-erythro-β-methylaspartic acid at position 3, ADDA (3-amino-9-methoxy-2.6.8-trimethyl-10-phenyldeca-4.6-dienoic acid) at position 5 and N-methyldehydroalanine (MDha) at position 7. Structural variations have been reported in all seven AAs, but the most frequent are the substitution of L-AA at position 2 and/or 4 and methylation/demethylation of AA at position 3 and/or 7 (Table 1). The name of MCYSTs variants are derived from these substitutions. For example, MCYST-LR contains two L-AA: leucine (L) and arginine (R), MCYST-YR contains tyrosine (Y) and arginine (R) while MCYST-RR contains two arginines (R) [34].

3.1. Gene cluster and MCYSTs biosynthesis

The MCYSTs are produced non-ribosomally by a hybrid multienzyme complex. This complex consists of polyketide/peptide synthetase and the tailoring enzymes. Both polyketide and peptide synthetases catalyze the condensation of simple metabolites in order to form complex secondary metabolites. They have a modular organization, with each module carrying the information for recognition, activation and modification of one substrate (e.g. amino acids for peptide synthetases) into the growing chain. Each module comprises domains responsible for a specific biochemical reaction [30]. The MCYSTs production occurs through the so-called thio-template mechanism found in bacteria and lower eukaryotes [2]. The best-characterised example of this mechanism is the synthesis of gramicidin S and tyrocidin in *Bacillus* [119] [128].

The genes encoding the multienzyme complex involved in MCYSTs synthesis are clustered together forming the *mcy* gene cluster [48]. The entire gene cluster and flanking regions, spanning more than 55 kb (*mcy* A-J) of the *mcy* region of *Microcystis* strain PCC 7806

was initially characterised by Tillett *et al*. [190]. At about the same time, sequencing of the microcystin biosynthesis gene cluster in a different *Microcystis* strain (K139) was reported [147, 148]. Recently, the *mcy* gene cluster of other cyanobacteria strains, such as *Planktothrix* strain CYA 126 [35] and *Anabaena* strain 90 [168] were also characterised. The pattern organisation of the gene clusters shows little variation among cyanobacterial genera [50]. Even the nodularin synthetase gene cluster has a high degree of sequence similarity to the microcystin synthetase gene cluster, a fact that suggests their homology [139]. It seems that nodularin synthetase gene set is originating from an ancestral microcystin synthetase gene set through deletion of two modules, parts of the *mcy*A and *mcy*B gene and by mutation(s) in *mcy*A, which change the substrate specificity [161]. Therefore, it was proposed that nodularin should be regarded as a structural variant of microcystin [161].

The first *mcy* cluster characterised, however, was that of the *Microcystis* genus. It comprises two polycistronic operons (*mcy* A-C and *mcy* D-J) transcribed from an internal bidirectional promoter as shown in Fig.1.

The operons consist of six large open reading frames (*mcy*A-E and G) and four small open reading frames (*mcy*F and H-J): *mcy*A, B and C genes encode peptide synthetases (comprising five modules), *mcy* D encodes a bimodular polyketide synthetase and *mcy*E and *mcy*G, two hybrid enzymes consisting of peptide synthetase and polyketide synthetase modules [190]. The small *mcy*J, *mcy*F and *mcy*I encode enzymes putatively involved in the tailoring [190]. The gene *mcy*H encodes a supposed ABC transporter [152]. As described by Tillett *et al.* [190] the biosynthesis of MCYSTs starts with ADDA formation and its linkage to D-glutamate. The enzymes encoded by the *mcy*D-J genes accomplish this. The next step, achieved with enzymes encoded by the genes *mcy*A, *mcy*B and *mcy*C, comprises the extension of the dipeptidyl intermediate to the heptapeptidic cyclic structure (Fig.1). By comparison of 19 *Microcystis* strains, by means of phylogenetical analysis, several genetic variations were noticed within the *mcy*B gene [134]. These differences are probably the result of recombination events between the sequences of *mcy*B and *mcy*C genes [134]. The same authors found the variations in the *mcyB* gene to be highly correlated with the production of certain MCYSTs congeners. Therefore, it is proposed that these genes play a role in the activation of the variable L-AA(s) of the MCYSTs.

The role of peptide synthetase genes in MCYSTs production was characterized by knockout experiments [48]. Further studies have shown that the difference between toxic and non-toxic *Microcystis* strains is the presence or absence of the *mcy*B gene [9, 48] and probably other *mcy* genes such as *mcy*A and *mcy*D [48, 147, 191]. Chorus and Bartram [34] confirmed that very few non-toxic *Microcystis* strains posses the *mcyB* gene. It is not know whether the *mcyB* gene is down regulated or mutated in these strains. Another possibility advanced by these authors is that these non-toxic strains contain a gene very similar to the *mcyB* gene that might be involved in the biosynthesis of another peptide. Recent studies suggest that microcystin synthetase genes were originally present also in the now non-toxin producing strains and that the microcystin biosynthesis pathway is very ancient [161, 134]. Based on these findings, the MCYST biosynthesis genes were explored as tools for the molecular identification of potentially toxic cyanobacterial strains [47, 145]. Indeed, such studies confirmed that a *Microcystis* strain containing the intact microcystin gene cluster should be viewed as potentially toxigenic (toxin producing).

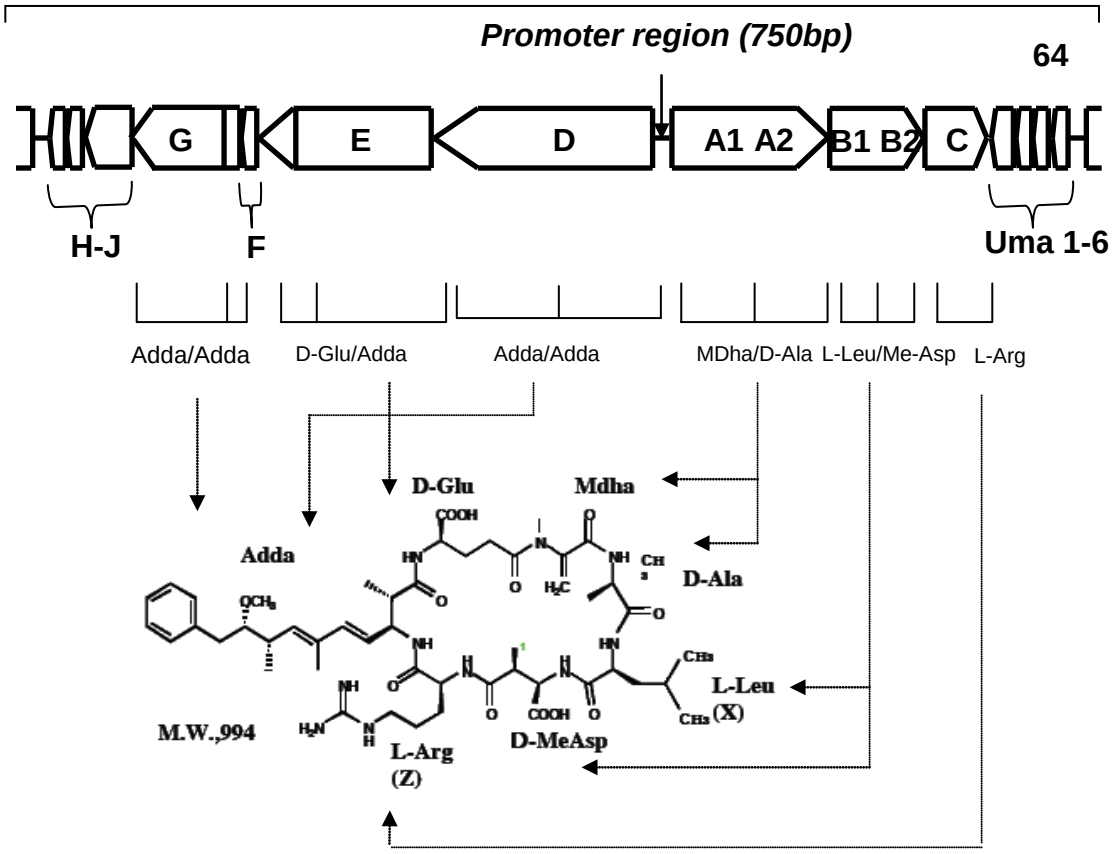


Fig. 1: In the upper section is presented the *mcy* gene cluster coding for the biosynthesis of MCYSTs in *Microcystis* (modified after [50]). The cluster consisit of: open reading frames (ORFs) containing regions coding to nonribosomal peptide synthetases (*mcy*A, *mcy*B and *mcy*C), to polyketide synthetases (*mcy*D) or to hybrid enzymes consisiting of combinated modules (*mcy*G and *mcy*E). Additional the cluster contains ORFs of putative microcystin tailoring function (*mcy*F, *mcy*I and *mcy*J), of non-microcystin synthetase ORFs (Uma 1-6) and the putative ABC-transporter ORFs (*mcy*H), respectively. In the following section, the synthetic model for formation of MCYST-LR is presented.

3.2. Environmental factors involved in MCYSTs production

A number of studies have been undertaken in order to establish the influence of environmental factors that lead to bloom formation and modulate the toxin production in cyanobacteria. The initial observation that freshwater cyanobacterial blooms form in eutrophic waters mostly during the summer seasons, when the temperature and the light exposures are high, was confirmed by laboratory work. For example, in *M. aeruginosa* culture the toxin production was found to increase proportionally with the intensity of light up to 40 microeinsteins/m^2/s but decreases at higher intensities [199]. These results were confirmed for several strains of *Oscillatoria agarhii* [178] and *Anabaena sp* [163]. For *Oscillatoria,* a direct relationship was found between nitrate and phosphorus concentrations, and toxin production [178]. An increase of intracellular toxins due to extracellular phosphorus concentrations was also recorded in two *Anabaena* strains [163] and for *Microcystis* [149, 211], suggesting that different cyanobacterial genera may respond similarly to the same external factors. The optimal temperature was established to be around 25°C for *Oscillatoria* [178], like in *Anabaena* [163] and between 20 and 24°C for *Microcystis* [201]. Interestingly, the toxin production seems to be limited by other factors such as zinc and iron [122, 200]. Despite a considerable pool of data collected up to now, more information is needed regarding the influence of the chemical and physical factors on toxin production and cyanobacterial growth in order to understand the dynamics of toxic blooms in nature.

3.3 The ecological role of MCYSTs

The exact ecological advantage gained by cyanobacteria due to MCYSTs production and release is as of yet little understood. A number of hypotheses have been put forward. Rohrlack *et al.* [167] have proposed a role of MCYSTs against zooplankton grazing. Sedmak and Kosi [175] and Pflugmacher [154] suggested that MCYSTs impact on proliferation and diversity of other phytoplankton species (allelopathic effect). MCYSTs may also act, under high light conditions, as extracellular signalling molecules in a quorum-sensing like mechanism required during the formation of a bloom [49]. Future work is required to fully understand the ecological role of these peptides. Overall, cyanobacterial secondary metabolites have been considered as powerful toxins affecting both aquatic (mussels, crayfish and various species of fish) and terrestrial organisms (locusts, birds and mammals). Indications for changes in ecosystem structure due to cyanobacterial toxins have been occasionally reported (e.g. for fish by [59]). These impacts, however, are unlikely to be a dominant advantage for cyanobacteria, because they are not direct competitors in an ecological sense with higher eukaryotic organisms, as mentioned above.

3.4. Toxic effects of MCYSTs on vertebrates

MCYSTs are essentially selective for liver. Once taken up by hepatocytes, they affect cell structure and functions by inducing oxidative stress and lipid peroxidation, DNA fragmentation, cytoskeletal disorganisation, loss of organelles and plasma membrane integrity, apoptosis and/or necrosis. Some of these effects are caused by MCYSTs' capacity to inhibit cellular protein phosphatases and to induce oxidative stress [78]. The *in vivo* reported outcome of acute exposure to MCYSTs are liver tissue disintegration, internal liver haemorrhage and animal death due to hemorrhagic shock (Fig 2).

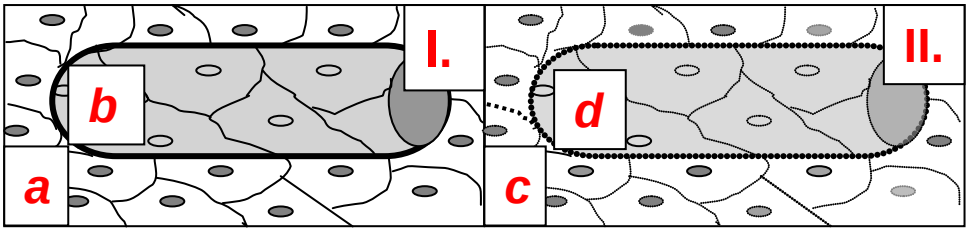


Fig. 2: I-Aspect of normal liver with intact hepatocytes structure (a) and capillary endothelial cells (b). II-Aspect of liver affected by MCYSTs with injured hepatocytes (c) and damaged sinusoid capillaries (d) (modified after [26]).

The MCYSTs variants differ in their toxic potential as determined by the conventional mouse bioassay. The reports indicate an LD_{50} value (lethal dose 50) that ranges from 50 to 1200 µg/kg body weight [34, 166]. For example, the MCYST-LR seems to have a lower LD_{50} value (50 µg/kg) than other variants, such as MCYST-RR and MCYST-WR, where values of 600 and 150-200 µg/kg body weight were reported [34].

The MCYSTs toxicity data reported in diverse studies are difficult to compare. A number of differences appear regarding LD_{50} for the same MCYST variant, both in *in vivo* and *in vitro* studies. These discrepancies can be explained by both species sensitivity and assay variability. In mice, for example, LD_{50}s for MCYST-LR were reported to range between 25 and 150 µg/kg of body weight [1]. Several studies in rats yielded an LD_{50} between 106 µg/kg and 122 µg/kg of body weight [138, 140]. Despite assay variability, fish have consistently been reported to be less sensitive than mammals. Fladmark *et al.* [71] observed by an *in vitro* study that salmon hepatocytes were 2 to 5 times less sensitive toward MCYST-LR than rat's hepatocytes. Sugaya *et al.* [184] found in an *in vivo* study that goldfish (*Carassius auratus*) was 30 times less susceptible than mice to an intraperitoneal (i.p.) injection of MCYSTs. Differences between fish species are also reported. For instance, rainbow trout is less responsive to MCYSTs when

compared to brown trout and carp [21, 186]. In mammals (mice) differences were also reported among different strains used for testing. Ito *et al*. [102] found Balb/C mice to be more responsive to oral MCYST-LR exposure than ICR mice. According to Lovell *et al.* [121], Balb/C mice were approximately two times more sensitive than Swiss Webster mice after i.p. exposure to MCYST-LR. Ito *et al.* [101] and Rao *et al*. [162] reported differences in sensitivity according to age despite identical strains of mice: older mice were more susceptible to MCYSTs. Other factors influencing susceptibility toward MCYSTs appear to be the physical state and sex. Miura *et al.* [138] called for caution when interpreting MCYSTs *in vivo* toxicity results, showing that the MCYST LD_{50} for fed rats was 122 µg/kg body weight while for fasted animals it was 72 µg/kg body weight. Similarly, *in vivo* and *in vitro* studies on goldfish identified a greater susceptibility toward MCYST-LR in fasted *versus* fed animals [125, 126]. *In vitro* studies pinpoint other differences related to the type of cell used, primary hepatocytes being the most sensitive [55, 57, 213], the type of culture [7], the medium used [188] and the time spent from hepatocytes isolation until their exposure [11].

Rao *et al*. [162] suggested that variation in response might be partly due to the source from where MCYSTs are purified or bought. Additionally, the different analytical laboratory methods need to be taken into account, since many laboratories purify and quantify these toxins "in house". An international comparison trial clearly showed discrepancies between 31 laboratories attempting to establish an accurate MCYSTs concentration in common samples [65] The study points out that even the same working group found 4 to 15% variation when individual results were repeated. Moreover, a 24 to 49% variation in reproducibility between all working groups was reported. These data emerged when the laboratories tested the agreed samples with identical toxin standard (provided at the same time as the samples). In the same study, by using their own laboratory standard (bought or manufactured "in house") further variation existed (up to ± 30%). These results clearly showed that, beside variation induced by the analytical protocols used, the quantification of the toxin might be influenced by the variability of available standards. Fastner *et al*. [65] assumed an equal value of about 30% error in quantification of MCYSTs in their experiments. Other authors stress the possibility that the toxins might be lost from aqueous and methanol stock solutions kept in glass or plastic vials. In such a study, the toxin concentration dropped to 32% as compared to the initial concentration [99]. Some researchers point out that the MCYST referred to in most of the studies as reference standards is not actually sold as such and, thus, the provided amounts cannot be guaranteed [130]. These variations in MCYSTs quality and quantity ultimately influence the conclusions drawn from toxicity studies. Chernoff *et al*. [32] found that two different lots of MCYST-LR obtained from the Sigma Company, led to different levels of toxicity. Using the same mice strain (CD1), LD_{50}s (i.p.) for the first and second MCYST-LR lot were 127 µg and 64 µg per body weight, respectively. Taken together, these data clearly show the weaknesses of the analytical and toxicological investigations in assessing the complete health impact due to MCYSTs. They emphasise the need for the production of a certified reference MCYST to be used by all laboratories, and an international operating procedure for standardised experiments.

3.5. The MCYSTs uptake in hepatocytes

The selectivity of MCYSTs for the liver can be explained by the active uptake of MCYSTs *via* the transport system for bile salts, which constitute an important fraction of the organic anions excreted by bile. Uptake of bile salts in mammalian liver occurs through the baso-lateral plasma membrane *via* sodium-dependent (Na^+ -taurocholate cotransporting polypeptides, NTCP) transporters and sodium-independent (organic anion-transporting polypeptides, OATPs) transporters [202]. In contrast, in liver of fish, such as *Raja erinacea* (little skate: [16, 75]) and *Oncorhynchus mykiss* (rainbow trout: [158]), the NTCP transporters are absent. The sodium independent transport system (OATPs transporters) seems to have a wide substrate spectrum.

Along with bile salts, bile acids, anionic diagnostic dyes, uncharged steroids and various linear and cyclic peptides (such as MCYSTs) may be transported.

So far, 36 Oatps (in rodents)/OATPs (in humans) have been identified [84]. The OATPs are currently classified within the solute carrier family (SLC21/SLCO), sharing as common features the 12 transmembrane-spanning topology and the large extracellular loop between transmembrane domains 9 and 10 [84]. OATPs share at least 40% amino acid sequence identity [202]. Dissimilar expression of OATPs in the gastrointestinal tract, liver and kidney, as well as at the level of the blood-brain barrier, has important implications for MCYSTs penetration. Their presence in the intestinal cells explains how MCYSTs pass the microvillus border upon oral intake [42, 62, 102] or by accidental ingestion [24]. A similar assertion is the basis of kidney damage observed in fish [68] and rats [136, 137] following MCYSTs exposure. The degree of uptake and the subsequent toxic effects varied with the cell types since the diverse OATPs isoforms do not have identical uptake rates and substrate specificity. A low level of MCYSTs is sufficient for a response in primary hepatocytes [57] whereas only at very high levels, the toxins are able to act as well on permanent cell lines [33, 155, 156, 218]. Along with primary hepatocytes and permanent cell lines, the blood cells, such as lymphocytes [117] or polymorphonuclear leukocytes [114] have been shown to be relatively sensitive to MCYSTs. The blood cells and tested permanent cell lines frequently lack the specific transporters [16, 54], therefore the probable mechanism of uptake implied here is different and may not involve the OATPs. Some of the MCYSTs congeners, such as MCYST-LF and -LW are more hydrophobic than MCYST-LR and might cross cell membranes by diffusion [206].

An involvement of OATPs in MCYST uptake was initially suggested in mammals because of the protection elicited against MCYSTs toxicity by known OATP inhibitors (*in vitro* [58, 170, 172, 189] and *in vivo* [115])**.** One such inhibitor is rifampicin, which has been shown to inhibit human OATP-C and rat Oatp4 [192, 204]. A recent study on MCYST uptake [70] clearly confirmed the link between OATP and MCYST-LR uptake both in humans and in rodents. The importance of bile/organic anion transporters in MCYSTs uptake was also identified in fish, particularly in rainbow trout liver [187] and gut [22], but a specific transport protein has not yet been ascribed.

In hepatocytes, MCYST uptake through OATPs quickly reaches a plateau [57]. Thereafter, lower amounts of MCYSTs are transported inside the cells, probably either due to saturation of the transport system [189] or to some other cellular effect that may inhibit further uptake [170, 172].

4. Mechanisms of MCYSTs action

4. 1 Phosphatase inhibition

After entering a cell, MCYSTs interact with several protein phosphatases (PPs) and inhibit their regular action. PPs are categorised in two major subtypes (PP1 and PP2) originally distinguished by their specificity for the phosphorylase kinase enzyme. PP1 dephosphorylates phosphorylase kinase β-subunit and PP2 dephosphorylates its α-subunit. PP2 are further divided into three subtypes: 2A, 2B and 2C, according to their requirement for divalent cations. Recent studies have shown that the amino acid sequences of PP1, PP2A, PP2B are related. They share about 49% sequence similarity in their catalytic subunits [92]. PP2C is structurally distinct and belongs to a separate gene family [38, 209]. Multiple isoforms of PP1, PP2A, PP2B and PP2C are present in mammals [92].

In vivo and *in vitro* studies indicate MCYSTs as inhibitors of cytoplasmic PP 1 and 2A in many phyla [87, 123, 171]. Beside cytoplasmatic PP, MCYSTs also target nuclear PP [82, 83]. MCYSTs seem to have no inhibitory potency on similar PPs found in cyanobacteria [177]. The IC_{50} (inhibition concentration 50) for either PP1 or PP2A is about 0.1-1.0 nM as determined for MCYST-LR, -LA and -YR in a standard inhibition assay [91, 96, 123]. Despite the apparent

specificity of MCYSTs for PP1 and PP2, some effect on other protein phosphatase isoforms, like PP4, PP5 and PP2B, at higher MCYSTs concentrations cannot be ruled out [87].

The nature of interaction between MCYSTs and PPs has been thoroughly described and the crystal structure of PP1 in a complex with MCYS-LR has been reported [79]. This interaction occurs in two steps [41] with the ultimate result being the blocking of the active centre of the PP enzymes by the MCYSTs. Three distinct regions of PP are involved in the interaction: the hydrophobic groove, the metal binding site and the edge of the C-terminal groove near the active site [79]. The high affinity of MCYSTs for PP1 is probably due to the similar conformation of the toxin in solution and in the toxin-enzyme complex [4]. Gulledge *et al*. [80] reviewed the PP and MCYSTs interaction. Some specific structure of MCYSTs (Tab. 2) relating to ADDA chain, Mdha and iso-linked D-glutamate seems to be necessary for their toxicity, particularly for the inhibition of PP [80, 86, 166, 183].

Table 2: Variation in MCYSTs associated with reducing their biological activity (after [80])

Residue present in MCYST-LR	Variations that eliminate toxicity
ADDA	(6Z)-ADDA
Mdha	N-Methyllanthionine
IsoGlu	IsoGlu-OMe IsoGlu-OC_2H_3(Me)OH

The general role of PPs in the cells is to antagonise the action of protein kinases (PKs). PKs phosphorylate the target proteins at specific sites (serine, threonine, tyrosine). The phosphate group is subsequently removed by the action of specific PPs. In eukaryotic cells, phosphorylation/dephosphorylation cycles of proteins is a key mechanism to control a multitude of cellular processes such as cell division, metabolism, contractility, membrane transport, transcription and translation. The balance of phosphorylation/dephosphorylation is tightly regulated. This balance can be severely disturbed by substances that have either PP or PK as a target [92].

4.2. Tumour promotion as a consequence of sublethal microcystin-induced protein phosphatase inhibition

Since the PPs play an important role in cell division, their inactivation by MCYSTs may affect cell proliferation and the homeostatic balance between cell proliferation and cell death. It is well known that PP2A is a major negative regulator of several steps in the MAP kinase (mitogen activated protein kinase, MAPK) signaling pathway which regulates the transcription of genes that are activated during cell proliferation [97]. It was shown that MCYST treatment can cause transformation (initial step in carcinogenesis) of immortalized colorectal crypt cells through activation of the MAPK pathway [219]. MAPKs are involved in a wide range of cellular responses, including inflammation and apoptosis. Thus an effect on the MAPK signaling pathway might produce two contradictory effects: in one way, the cell proliferation and tumor promotion and in a second way, apoptosis. In support of the idea that MCYSTs have tumour promoting potential stands their reported effect on p53 proteins. The p53 protein is a transcription factor that regulates the expression of a wide variety of genes involved in cell cycle arrest and apoptosis in response to genotoxic or cellular stress. More precisely, p53 arrests cell growth when some stress conditions are acting on or within the cell that are able to produce injures (e.g. DNA damage). Then, by arresting the cell cycle, p53 protein allows the repair of damaged DNA before the cells undergo replication. When the harm is too severe, p53 proteins

induce apoptosis. If p53 is not operational, the damaged cell may not be arrested to restore the injuries or the programmed cell death might not occur [6]. The result is unstoppable proliferation of these damaged cells that might further develop into a tumour. The p53 is a phosphorylated protein, its phosphorylation /dephosphorylation status being controlled by several PK and PP. For example, it is known that p 53 is dephosphorylated by the PP2A. Thus, PP2A inhibition by MCYSTs may further result in alteration of p53 function. Indeed, some studies on mouse cells exposed to MCYSTs report the hyperphosphorylation of p53 and an increment of their expression level, followed by apoptosis [76, 83].

The dualistic involvement of MCYSTs in apoptosis and in tumour promotion is difficult to comprehend. As reviewed by Gehringer [78], these dualistic responses originate in the MCYSTs doses used, being difficult to draw an exact border. Okadaic acid, an inhibitor of PPs acting in the same manner as MCYSTs, is also capable of inducing tumours [77] as well as apoptosis [160]. Thus, exposure to low doses of MCYSTs may possibly result in an enhanced proliferation of cells [94] which may cause tumour promotion in humans, as suggested by the cases of hepatic cancer reported after sublethal, prolonged exposure to MCYSTs [216]. Exposure of cells to high MCYSTs doses might result in apoptosis (see 4.3). One early study showed that ingestion of a *Microcystis* extract promoted skin tumours in mice after initiation by exposure to a carcinogen (dimethylbenzanthracene) [61]. Single i.p. injection of diethylnitrosamine followed by i.p. MCYST-LR injections over several weeks induced preneoplastic liver foci and nodules in rats [146]. Different studies have shown the formation of neoplastic liver nodules in mice exposed to repeated i.p. injections of MCYST-LR (20µg/kg, 100 injections over 28 weeks) without any pre-treatment with initiators [101]. More recently, Humpage *et al.* [95] showed that after i.p. injection with the carcinogen azoxymethane, followed by MCYST exposure, an increase in the number of aberrant crypt foci in the mouse colon occurred.

4.3. Cellular effects of microcystin-induced protein phosphatase inhibition

Disruption of the cytoskeleton. Covalent binding to, and inhibition of, PPs by MCYSTs lead to hyperphosphorylation of cellular proteins followed by disturbance of a number of important cellular mechanisms.

Two significantly affected proteins are the K8 and K18 keratins [194, 195], which are the only cytoskeletal intermediate filament proteins in hepatocytes. MCYST treatment also produces hyperphosphorylation of desmoplakin I and II (desmosomal linking proteins) [194]. These findings confirm the results of Stappenbeck *et al*. [182], who showed that keratins directly interact with desmoplakin in a phosphorylation-dependent way. MCYST-LR also cause hyperphosphorylation of dynein [173]. Dynein is a motor protein associated with microtubules and involved in the transport of organelle along the microtubular pathways of the cytoskeleton [106]. The enzymes responsible for phoshorylation of these cytoskeletal proteins were assumed to be protein kinase C, protein kinases A or calcium/calmodulin-dependent protein kinases II [194]. Since the PPs necessary to reverse the action of the protein kinases (PK) are inactivated results unabated activation of the PK enzymes and overall hyperphophorylation of the proteins. Other groups [56, 194, 121] found that MCYSTs also induce the reorganization of the microfilaments (e.g. F-actin) and the time-dependent disassembly of the microtubules. The net result of MCYSTs action is the complete disruption of hepatocyte cytoskeleton, with an impact on the intracellular distribution and shape of organelles, such as the ER [120] and the lysosomes [11, 118, 155, 156]. This also affects the intracellular transport [85, 173] as well as processes such as endocytosis or autophagy [90]. The ultimate step is cell deformation and loss of cell-cell adhesion contacts. As a result, the normal liver architecture breaks down, leading to massive hepatic haemorrhage and animal death [24, 26, 82].

Apoptosis. Despite numerous reports on MCYSTs toxic effects, there is no clear picture of the exact mechanism of their toxicity. There is no agreement yet on what type of cell death

these toxins elicit - apoptosis or necrosis. Apoptotic or necrotic death seems to be, at the first glance at least, two disparate extremes. Largely, the distinction is based on morphological features, particularly the ability of the cells to maintain the integrity of the plasma membrane. Necrotic cells disassemble and release their membrane compounds (e.g. derivates of membrane phospholipids, such as arachidonic acid). The extracellular presence of these molecules constitutes a signal for the neighbouring cells, which react to defend themselves, with the overall induction of the inflammatory response. Conventionally, cell lysis and the inflammatory response are features specific to necrosis. However, in some cases, the apoptotic cells may undergo secondary necrosis during which the cell lyses and might produce an inflammatory response, which makes it difficult to catalogue the type of cell death. It is generally accepted that MCYSTs induce primarily the apoptotic events. The necrosis [69, 101] and the inflammatory reaction (see 4.6.) reported in a number of studies appear to be, more probably, secondary responses.

Several *in vitro* studies revealed that MCYSTs appear to be able to initiate apoptosis [12, 45, 129]. Results were supported by reactions, such as membrane blebbing, cell contraction, externalization of membrane phosphatidyl serine, chromatin condensation and intranucleosomal DNA fragmentation. The *in vitro* findings also were confirmed *in vivo,* upon i.p. administration of MCYST-LR induced apoptosis in rats and mice [31, 82, 93, 181]. Based on toxin doses used, two independent pathways, involved in MCYSTs induced apoptosis, were proposed by [31]. The lower toxin doses initiate the mitochondrial pathway trough the pro- and anti-apoptotic proteins BAX-BCL2-BID. Higher toxin doses can cause apoptosis via the reactive oxygen species pathway. It is difficult to draw a strict border between these two proposed pathways, and to study their interconnection is a very important perspective. The apoptotic mechanism of MCYSTs is currently an active area of research.

The BAX-BCL2-BID pathway. A number of recent studies demonstrate that MCYST-LR induces apoptosis trough the involvement of BAX-BCL2-BID proteins and mitochondria [31, 76]. Mitochondria are a vulnerable target for many different toxins [207]. BCL2 is an anti-apoptotic protein located in the endoplasmic reticulum membrane and in the outer mitochondrial membrane. BID and BAX are cytosolic pro-apoptotic proteins that are activated by diverse cell death signals. After activation through dephosphorylation or cleavage, they localize to the mitochondrial surface and interact with BCL2 disrupting its normal function and leading to the formation of pores in the mitochondrial membranes [20]. The newly formed holes allow the release of cytochrome *c* and other pro-apoptotic molecules into the cytosol. These molecules lead to activation of caspase or calpain pathways and finally to apoptosis. The cell sensitivity towards apoptotic stimuli depends on the balance of these pro- and anti-apoptotic proteins. An excess of pro-apoptotic proteins makes the cells more sensitive to apoptosis, whereas an excess of anti-apoptotic proteins leads to more resistant cells. Transcriptomic and proteomic analyses in mice showed that the level of BCL2, BAX and BID proteins seems to increase as a result of toxin treatment [31]. Related findings were reported in rats [76]. These authors found that MCYST-LR exposure can increase the expression of p53 and of BAX proteins both *in vivo* and *in vitro*. The level of BCL2 seems to decrease only *in vitro,* with no differences observed under *in vivo* conditions. Both studies concluded the involvement of these proteins in the regulation of MCYSTs induced apoptosis. The participation of mitochondria in MCYST induced apoptosis is also supported by some other studies. For example, a rapid decrease of mitochondrial membrane potential and changes in mitochondrial permeability transition was shown after toxin treatment [44]. The membrane potential is essential for the proper functioning of mitochondria, particularly in the process of oxidative phosphorylation. Alterations of this potential may indicate some functional alterations of mitochondria and might be a cause of MPT (mitochondrial permeability transition) pore formation. The formation of such a pore complex was demonstrated through

increased permeability of the mitochondrial inner membrane following MCYST exposure [45, 46]. After the opening of MPT pore, the cytochrome *c* and Ca^{2+} are released from the mitochondria and further activate specific caspases, the Ca^{2+}-dependent protein kinases or the calpains, which in turn determine the cell to enter apoptosis. The cytochrome *c* release [46] and calpain activation [13] have been reported to occur subsequently to MCYST treatment. Furthermore, a role of calcium/calmodulin-dependent protein kinase II in the apoptotic process due to MCYST action has been assumed [72, 112]. Its activation is based on a mitochondrion-mediated release of Ca^{2+} due to toxin treatment and on cellular protein hyperphosphorylation as a response to PP inhibition. The increase of cytoplasmatic Ca^{2+} level in the treated hepatocytes has been previously shown by Runnegar *et al*. [170]. The involvement of protein kinases (PKs) in MCYSTs apoptosis is supported by Blankson *et al*. [10] who showed that naringin, a PK inhibitory flavonoid, can protect the hepatocytes against toxin-induced apoptosis and also against hyperphosphorylation and disruption of the keratin cytoskeleton network. Pre-treatment with naringin has been shown to protect mice against a lethal dose of MCYST-LR [115]. The manner of PK action in apoptosis induction is still under investigation. Mikhailov *et al*. [135] proposed a different pathway for MCYST-induced apoptosis. By means of specific antibodies for MCYST, they demonstrated that the toxin, at high concentration, is able to bind to the β-subunits of the ATP-synthetase. They hypothesized that this binding may act as a signalling target for apoptosis, leading to the disturbance of mitochondrial function and the release of cytochrome *c*.

The pathway involving the oxidative stress. As previously mentioned, the second pathway involved in MCYSTs induced apoptosis is related to oxidative stress and reactive oxygen species production. Oxidative stress is defined as an exposure of cells or tissues to excessive level of oxidants, particularly, the superoxide or hydroxyl free radicals. These radicals are termed reactive oxygen species (ROS). The oxidative stress can be triggered by a high production of ROS, a low level of cellular antioxidants, or both. Both factors seem to be involved in MYCST induced oxidative stress. ROS production (determined by changes in H_2O_2 and O_2^- concentrations) followed by lipid peroxidation and reduction of glutathione levels have been confirmed consecutive to MCYST exposure [15, 198]. It is well established that ROS can promote the opening of the mitochondrial MPT pore [39] and trigger the apoptotic events. Conjugation of MCYSTs with glutathione, as shown by Kondo *et al.* [109, 110] and Takenaka [185], plays an important role in the metabolism and detoxification of MCYSTs [153] (Fig. 3). Collectively, glutathione- and cysteine-derivatives of MCYSTs have been found to be less toxic than MCYSTs themselves, as evidenced by an LD_{50} of 38 µg/kg for parent MCYST-LR, 630 µg/kg for MCYST-LR-GSH and 267 µg/kg for MCYST-LR-Cys, respectively [103].

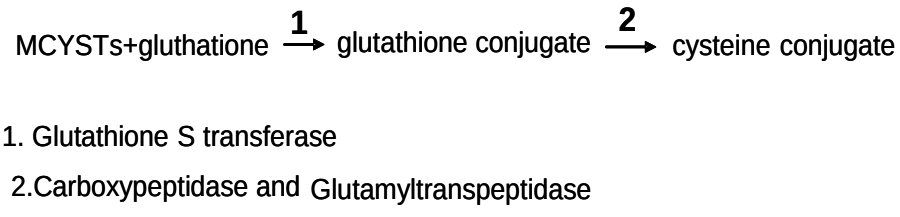


Fig. 3: The MCYSTs detoxification steps involving enzymes of phase II detoxification pathway. This is called the conjugation pathway: glutathione and other molecules are added to a toxic chemical or drug to make it more water-soluble, so it can be excreted from the cells thus be detoxified (after [176]).

Other glutathione-dependent detoxification enzymes, such as glutathione peroxidase and glutathione reductase, as well as superoxide dismutase and catalase, have been shown to be decreased in liver and kidney of rats exposed to MCYST [140]. Beside oxidative damage to lipids *via* lipid peroxidation, ROS can also induce oxidative damage of DNA [40]. Oxidative

damage of DNA caused by MCYSTs-induced oxidative stress has been reported [15, 217]. In addition, Mankiewicz *et al*. [127] reported genotoxicity following exposure to cyanobacterial extracts containing MCYSTs. The DNA damage is normally reversed by DNA repair mechanisms involving processes such as base and nucleotide excision pathways. Unrepaired oxidative DNA damage can lead either to mutation and transformation of cells into malignant ones [40], or might activate the p53 pathway and subsequently trigger apoptosis [214].

Earlier *in vivo* studies have shown that antioxidants, such as E vitamin, silymarin and glutathione can provide protection against MCYSTs induced toxicity in mice and rats [88, 132]. Silymarin and N- acetylcysteine (a glutathione precursor) protect against MCYSTs toxicity *in vitro* as well. They attenuated lactate dehydrogenase release, cell detachment and the disruption of the cytoskeleton [44, 45, 131]

Nucleus related effects. MCYSTs have been shown to inhibit protein synthesis *in vitro,* without interfering with the amino acid transport [36]. Guzman and Solter [82] confirmed this effect by showing that total mRNA levels decline in MCYSTs treated cells. These inhibitory effects might be explained by a direct impact of the cyanotoxins on nuclear functions. Some reports indicate that MCYSTs can accumulate in the nucleus [69, 215] and covalently bind the nuclear PP [83]. Repavich *et al*. [164] showed that, along with nuclear accumulation, the purified toxins have a clastogenic (microscopically visible damage or change to chromosomes) response on human lymphocytes, seen as a dose-related increase of chromosomal breakage. More recently, it has been shown that a cyanobacterial extract containing MCYSTs had a strong mutagenic effect as measured by the Ames test (with and without S9 activation). The extract induced DNA damage in primary rat hepatocytes and micronucleated polychromatic erythrocytes from the bone marrow of mice [44]. Decordier *et al.* [43] showed that the micronucleated cells or cells that acquired chromosomal aberrations could strongly trigger the apoptosis. Thus, these additional findings sustain the role of MCYSTs in inducing apoptotic phenomena.

4.4. Possible reproductive and developmental effects

In vivo experiments carried out on mice have shown that the administration of MCYSTs (approximately 2800 µg/kg body weight/day) for 17 weeks prior mating, throughout pregnancy and during early lactation, have no effect on fertility, embryonic mortality or teratogenicity but reduce the brain size of neonatal mice by about 10% as compared to controls [60]. The gavage administration of pure MCYST-LR to mice (up to 2000 µg/kg body weight/day) from day 6 to day 15 of pregnancy evidenced retardation of foetal weight and of skeletal ossification. No effects of the toxin on sex ratio, implantation, post- implantational loss or visceral/skeletal abnormalities were registered [66]. No evidence was found that MCYST-LR (up to 100 µM) affects the *in vivo* embryonic, foetal or postnatal development in the mouse or the *in vitro* embryonic development [32] or rabbit [74]. This lack of toxicity was assumed to be due to a lack of MCYSTs uptake through the placenta or the generally limited sensitivity of embryonic tissues to these toxins. The effects of MCYSTs on embryonic development of fish are controversial due to differences in the embryos membrane permeability between fish species. To avoid this problem it was suggested to inject the MCYSTs directly into embryos. Exposure of embryo of medaka fish (*Oryzias latipes*) to up 10 µg/ml MCYST-LR by microinjection resulted in 90% mortalities. Embryos also exhibited hepatobiliary abnormalities like hepatic hemorrhage and increasement of the liver size [104]. Various abnormalities and lethality was reported also for zebrafish (*Danio rerio*) embryos microinjected with MCYST-LR (900 nM intracellular concentration [210].

4.5. Other reported effects

In addition to liver damage, Le Claire *et al*. [119] observed certain effects of MCYSTs on the circulatory system in rats. The various noticed effects included a decreased heartbeat, followed by acute hypotension and peripheral vasoconstriction.

MCYSTs decreased the glycogen levels in the livers of treated mice [133, 138]. These results correlate with the data of Runnegar *et al*. [169] who observed an activation of glycogen phosphorylase A. In addition, Guzman and Solter [82] reported the inhibition of glycogen synthetase following MCYSTs treatment. This activation/inhibition process can easily be interpreted by protein hyper-phosphorylation elicited via PP inhibition due to MCYSTs. The glycogen synthetase enzyme is inhibited by phosphorylation whereas glycogen phosphorylase, which hydrolyses the glycogen to glucose phosphate, is stimulated by phosphorylation [100], the final result being liver glycogen breakdown.

Studies focused on macrophages indicate that they are susceptible to MCYST exposure as well, thus suggesting a role of white cells in MCYST-induced progressive liver damage and tumour promotion. For example, macrophages responded to MCYSTs exposure by producing interleukin-1 (IL-1), and tumour necrosis factor-α (TNF-α) [113, 142, 143]. Naseem et al. [144] showed that MCYSTs induce the release of arachidonic acid and prostaglandins in hepatocytes. IL-1 and TNF-α are pro-inflammatory cytokines involved in the acute phase response of the liver. Prostaglandins are highly potent substances (hormone-like compounds), not stored but produced as needed by cell membranes in virtually every body tissue. The prostaglandins are formed following the oxygenation of arachidonic acid (a polyunsaturated fatty acid) through the cyclooxygenase (Cox) pathway. It is known that the releases of arachidonic acid and of prostaglandins are steps in activation and modulation of inflammatory responses. Thus, the compounds that are characteristic for inflammed tissues may act, together with PP inhibition, in the promotion of liver tumours.

5. Conclusions

The data summarised in this review emphasise the risk raised by cyanobacterial proliferation in relation to their ability to produce and release powerful toxins. Once freed in the water, these toxins may harm both aquatic environment and terrestrial organisms, via accidental ingestion, inhalation or by dermal contact [51]. Depending on the route of exposure and on the level of cyanotoxins in the organism, diverse outcomes can emerge. The worst scenarios are those ending in animals' death, a fact many times reported in the case of cyanotoxins [63]. In several countries, governmental and non-governmental organizations initiated monitoring programs to assess the cyanobacterial occurrence and the risk of human exposure. Obviously, the field of cyanotoxin research has to be extended to cover a much wider range of the toxin variety. Until now, the research was mostly focused on microcystins. Despite this, not all of the MCYST toxic mechanisms were deciphered and many reported effects are contradictory. Initial steps have to be taken in regard to standardization of the toxicity experiments and the establishment of a certified reference for MCYSTs. Moreover, most of the data concern MCYST-LR, a fact that may artificially overemphasise the importance of this variant. Up to now more than 80 MCYSTs variants were described and most of them are as of yet not tested for their toxicity. Testing the potential toxic effect of other variants may help us to understand the toxicity of extracts that normally contain more than one MCYST variant. In natural conditions, a cyanobacterial bloom produces diverse MCYSTs, which might interact in potentiating or inhibiting their action.

Some other directions would be the investigation of MCYSTs toxicity towards more directly exposed organisms that reside within the same aquatic environment. The following issues need to be addressed: what mechanisms do these organisms possess to protect their PP against MCYSTs? Why are the toxins produced? Why are they so diverse? Why are they produced by some species or strains but not by others? Every day, more information is collected

but more research is needed to fully understand the mechanisms of action and the potency of the cyanobacterial toxins.

Acknowledgements: We are grateful to Corina Luminița ROȘIORU, Horia BANCIU and to Manuela BANCIU for their critical reading of this manuscript.

REFERENCES

1. *** Toxic Cyanobacteria in Water 1999, *A Guide to Their Public Health Consequences*, Monitoring and Management, WHO.
2. Arment, A.R., Carmichael, W.W., 1996, Evidence that microcystin is a thio-template product, *J. Phycol*., **32**: 591-597.
3. Aronstam, R.S., Witkop, B., 1981, Anatoxin-A interactions with cholinergic synaptic molecules, *PNAS*., **78**: 4639-4643.
4. Bagu, J.R., Sönnichsen, F.D., Williams, D., Andersen, R.J., Sykes, B.D., Holmes, C.F.B., 1995, Comparison of the solution structures of microcystin-LR and motuporin, *Struct. Biol.*, **2**: 114-116.
5. Baker, P.D., Humpage, A.R., 1994, Toxicity associated with commonly occurring cyanobacteria in surface waters of the Murray-Darling Basin, Australia, *Aust. J. Mar. Freshwater Res*., **45**: 773-786.
6. Bates, S., Vousden, K.H., 1999, Mechanisms of p53-mediated apoptosis, *Cell. Mol. Life. Sci.*, **5**: 28-37.
7. Battle, T., Maguire, T., Moulsdale, H.J., Doyle, A., 1998, Progressive maturation resistance to microcystin-LR cytotoxicity in two different hepatospheroidal models, *Cell. Biol. Toxicol*., **15**: 3-12.
8. Bishop, C.T., Anet, E.F., Gorham, P.R., 1959, Isolation and identification of the fast death factor in *Microcystis aeruginosa* NRC-1, *Can. J. Biochem. Physiol*., **37**: 453-471.
9. Bittencourt-Oliveira, M.D., 2003, Detection of potential microcystin-producing cyanobacteria in Brazilian reservoirs with a mcyB molecular marker, *Harmful Algae*, **2**: 51-60.
10. Blankson, H., Grotterød, E.M., Seglen, P.O., 2000, Prevention of toxin-induced cytoskeletal disruption and apoptotic liver cell death by the grapefruit flavonoid, naringin, *Cell. Death. Differ*., **7**: 739–746.
11. Boaru, D.A., Dragoș, N., Schirmer, K., 2006, Microcystin-LR induced cellular effects in mammalian and fish primary hepatocyte cultures and cell lines: a comparative study, *Toxicology*, **218**: 134-148.
12. Bøe, R., Gjertsen, B.T., Vintermyr, O.K., Houge, G., Lanotte, M., Døskeland, S.O., 1991, The protein phosphatase inhibitor okadaic acid induces morphological changes typical of apoptosis in mammalian cells, *Exp. Cell Res.*, **195**: 237–246.
13. Botha, N., Gehringer, M.M., Downing, T.G., Van de Venter, M., Shephard, E., 2004, The role of microcystin-LR in the induction of apoptosis and oxidative stress in CaCo2 cells, *Toxicon*, **43**: 85-92.
14. Bouaicha, N., Maatouk, I., 2004, Genotoxic potential of microcystin-LR and nodularin in vitro in primary cultured rat hepatocytes and in vivo in rat liver cells, *Sixth International Conference on Toxic Cyanobacteria*, Bergen, Norway: 13–14.
15. Bouaicha, N., Maatouk, I., 2004, Microcystin-LR and nodularin induce intracellular glutathione alteration, reactive oxygen species production and lipid peroxidation in primary cultured rat hepatocytes, *Toxicol. Lett*., **148**: 53-63.
16. Boyer, J.L., Hagenbuch, B., Ananthanarayanan, M., Suchy, F., Stieger, B., Meier, P.J., 1993, Phylogenic and ontogenic expression of hepatocellular bile acid transport, *Proc. Natl. Acad. Sci.*, **90**: 435–438.
17. Brock, T.D., 1973, Evolutionary and ecological aspects of the cyanophytes. *The Biology of the Blue-Green Algae*, In: N.G. Carr and B.A. Whitton [Eds]. Blackwell Scientific Publications, Oxford, p. 487-500.
18. Brock, T.D., 1973, Lower pH limit for the existence of blue-green algae: Evolutionary and ecological implications, *Scienc*e, **179**: 202-203.
19. Bryant, D.A., 1994, *The Molecular Biology of Cyanobacteria*. Kluwer Academic Publishers, Dordrecht.
20. Burlacu A., 2003, Regulation of apoptosis by Bcl-2 family proteins, *J. Cell Mol. Med*., **7**: 249-257.
21. Bury, N.R., McGeer, J.C., Eddy, F.B., Codd, G.A., 1997, Liver damage in brown trout, *Salmo trutta* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), following administration of the cyanobacterial hepatotoxin microcystin-LR via the dorsal aorta, *J. Fish Dis.*, **20**: 209-215.
22. Bury, N.R., Newlands, A.D., Eddy, F.B., Codd, G.A., 1998, In vivo and in vitro intestinal transport of H-microcystin-LR, a cyanobacterial toxin, in rainbow trout (*Oncorhynchus mykiss*), *Aquat. Toxicol*., **42**: 139–148.
23. Carmichael, W.W., He, .J.W., Eschedor, J., He, Z.R., Juan, Y.M., 1988, Partial structural determination of hepatotoxic peptides from *Microcystis aeruginosa* (cyanobacterium) collected in ponds of central China, *Toxicon.*, **26**: 1213-1217.

24. Carmichael, W.W., 1992, Cyanobacteria secondary metabolites - the cyanotoxins, *J. Appl. Bacteriol*., **72**: 445-459.
25. Carmichael, W.W., Falconer. I.R., 1993, Diseases related to freshwater blue-green algal toxins, and control measures. In: *Algal Toxins in Seafood and Drinking* Water, Falconer, I. A. [Ed.]. Academic Press, p. 187-209.
26. Carmichael, W.W., 1994, The toxins of cyanobacteria, *Sci. Am*., **270**: 78-86.
27. Carmichael, W.W., 1997, The cyanotoxins, In: *Advances in Botanical Research. J.A. Callow,* [Ed.] Academic Press, London. **47**: 211-255.
28. Carmichael, W.W., Evans, W.R., Yin, Q.Q., Bell, P., Moczydlowski, E., 1997, Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont), *Appl. Environ. Microbiol*., **63**: 3104-3110.
29. Carmichael, W.W., Azevedo, S.M., An, J.S., Molica, R.J., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K., 2001, Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins, *Environ. Health Perspect*., **109**: 663-668.
30. Chang, Z., Flatt, P., Gerwick, W.H., Nguyen, V.A., Willis, C., Sherman, D.H., 2002, The barbamide biosynthetic gene cluster: a novel marine cyanobacterial system of mixed polyketide synthase (PKS)-non-ribosomal peptide synthetase (NRPS) origin involving an unusual trichloroleucyl starter unit, *Gene*, **296**: 235-247.
31. Chen, T., Wang, Q.S., Cui, J., Yang, W., Shi, Q., Hua, Z.C., Ji, J.G., Shen, P.P., 2005, Induction of apoptosis in mouse liver by microcystin-LR - A combined transcriptomic, proteomic, and simulation strategy, *Mol. Cell. Proteomics*, **4**: 958-974.
32. Chernoff, N., Hunter, E.S., Hall, L.L., Rosen, M.B., Brownie, C.F., Malarkey, D., Marr, M., Herkovits, J., 2002, Lack of teratogenicity of microcystin-LR in the mouse and toad, *J. Appl. Toxicol*., **22**: 13-17.
33. Chong, M.W.K., Gu, K.D., Lam, P.K.S., Yang, M., Fong, W.F., 2000, Study on the cytotoxicity of microcystin-LR on cultured cells, *Chemosphere*, **41**: 143-147.
34. Chorus, I., Bartram, J., 1999, *Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Managament*, E & FN Spon. London.
35. Christiansen, G., Fastner, J., Erhard, M., Börner, T., Dittmann, E., 2003, Microcystin biosynthesis in *Planktothrix*: Genes, evolution, and manipulation, *J. Bacteriol*., **185**: 564-572.
36. Claeyssens, S., Francois, A., Chedeville, A., Lavoinne, A., 1995, Microcystin-Lr induced an inhibition of protein-synthesis in isolated rat hepatocytes, *Biochem. J*., **306**: 693-696.
37. Codd, G.A., 2000, Cyanobacterial toxins, the perception of water quality, and the prioritisation of eutrophication control, *Ecol. Eng*., **16**: 51–60.
38. Cohen, P., 1989, The structure and regulation of protein phosphatases, *Annu. Rev. Biochem.,* **58**: 453-508.
39. Colell, A., García-Ruiz, C., Mari, M, Fernández-Checa, J.C., 2004, Mitochondrial permeability transition induced by reactive oxygen species is independent of cholesterol-regulated membrane fluidity, *FEBS Lett*., **560**: 63-8.
40. Cooke, M.S., Evans, M.D., Dizdaroglu, M, Lunec, J., 2003, Oxidative DNA damage: mechanisms, mutation, and disease, *FASEB J*., **17**: 1195-1214.
41. Craig, M., Luu, H.A., McCready, T.L., Williams, D., Andersen, R.J., Holmes, C.F.B., 1996, Molecular mechanisms underlying the interaction of motuporin and microcystins with type-1 and type-2A protein phosphatases, *Biochem. Cell Biol*., **74**: 569–578.
42. Dahlem, A.M., Hassan, A.S., Swanson, S.P., Carmichael, W.W., Beasley, V.R., 1989, A model system for studying the bioavailability of intestinally administered microcystin-LR, a hepatotoxic peptide from the cyanobacterium *Microcystis aeruginosa*, *Pharmacol. Toxicol*., **64**: 177-181.
43. Decordier, I., Dillen, L., Cundari, E., Kirsch-Volders, M., 2002, Elimination of micronucleated cells by apoptosis after treatment with inhibitors of microtubules, *Mutagenesis*, **17**: 337-344.
44. Ding, W.X., Shen, H.M., Zhu, H.G., Ong, C.N., 1998, Studies on oxidative damage induced by cyanobacteria extract in primary cultured rat hepatocytes, *Environ. Res.*, **78**: 12-18.
45. Ding, W.X., Shen, H.M., Ong, C.N., 2000, Critical role of reactive oxygen species and mitochondrial permeability transition in microcystin-induced rapid apoptosis in rat hepatocytes, *Hepatology*, **32**: 547-55.
46. Ding, W.X., Shen, H., Ong, C.N., 2002, Calpain activation after mitochondrial permeability transition in microcystin induced cell death in rat hepatocytes, *Biochem. Biophys. Res. Commun*., **291**: 321-331.
47. Dittman, E., Meissner, K., Börner, T., 1996, Conserved sequences of peptide synthetase genes in the cyanobacterium *Microcystis aeruginosa*., *Phycologia*, **35**: 62-67.
48. Dittman, E., Neilan, B.A., Erhard, M.V., Döhren, H., Börner, T., 1997, Insertional mutagenesis of a peptide synthetase gene which is responsible for hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806, *Mol. Microbiol*., **26**: 779-787.

49. Dittmann, E., Erhard, M., Kaebernick, M., Scheler, C., Neilan, B.A., Von Dohren, H., Borner, T., 2001, Altered expression of two light-dependent genes in a microcystin-lacking mutant of *Microcystis aeruginosa* PCC 7806, *Microbiology*, **147**: 3113-3119.
50. Dittmann, E., Borner, T., 2005, Genetic contributions to the risk assessment of microcystin in the environment, *Toxicol. Appl. Pharmacol*., **203**: 192-200.
51. Dittmann, E., Wiegand, C., 2005, Cyanobacterial toxins - occurrence, biosynthesis and impact on human affairs, *Mol. Nutr. Food Res*., **50**: 7-17.
52. Douglas, S.E., 1994, Chloroplast origins and evolution. In: *The Molecular Biology of Cyanobacteria*, Kluwer D.A. Bryant [Ed.]. Academic Publishers, Dordrecht, p. 91-118.
53. Elleman, T.C., Falconer, I.R., Jackson, A.R.B., Runnegar, M.T., 1978, Isolation, characterization and pathology of the toxin from a *Microcystis aeruginosa* (*Anacystiscyanea*) bloom, *Aust. J. Biol. Sci*., **31**: 209-218.
54. Enrique, C.T., Shapiro, R.J., Agellon, L.B., 1996, Reconstitution of bile acid transport in the rat hepatoma McArdle RH-7777 cell line, *Hepatology*, **1**: 206-211.
55. Eriksson, J.E., Hägerstrand, H., Isomaa, B., 1987, Cell selective cytotoxicity of a peptide toxin isolated from the cyanobacterium *Microcystis aeruginosa*, *Biochim. Biophys. Acta*, **930**: 304- 310.
56. Eriksson, J.E., Paatero, G.I.L., Meriluoto, J.A.O., Codd, G.A., Kass, G.E.N., Nicotera, P., Orrenius, S., 1989, Rapid reorganization of microfilaments induced in isolated rat hepatocytes by microcystin-LR, a cyclic peptide toxin, *Exp. Cell Res*., **185**: 86-100.
57. Eriksson, J.E., Toivola, D., Meriluoto, J.A.O., Karaki, H., Han, Y.G., Hartshorne, D., 1990, Hepatocyte deformation Induced by cyanobacterial toxins reflects inhibition of protein phosphatases, *Biochem. Biophys. Research Commun*., **173**: 1347-1353.
58. Eriksson, J.E., Grönberg, L, Nygård, S., Slotte, P., 1990, Hepatocellular uptake of 3H-dihydro-microcystin-LR, *Biochim. Biophys. Acta*, **1025**: 60-66.
59. Ernst, B., Hitzfeld, B.C., Dietrich, D.R., 2001, Presence of *Planktothrix sp*. and cyanobacterial toxins in Lake Ammersee, Germany and their impact on whitefish (*Corregonus lavaretus* L.), *Environ.Tox*., **16**: 483-388.
60. Falconer, I.R., Smith, J.V., Jackson, A.R., Runnegar, M.T., 1988, Oral toxicity of a bloom of the cyanobacterium *Microcystis aeruginosa* administered to mice over periods up to 1 year, *J. Toxicol. Environ. Health*, **24**: 291-305.
61. Falconer, I.R., 1991, Tumor Promotion and Liver Injury Caused by Oral Consumption of Cyanobacteria, *Environ. Toxicol. Water Qual*., **6**: 177-184.
62. Falconer, I.R., Dornbusch, M., Moran, G., Yeung, S.K., 1992, Effect of the cyanobacterial (blue-green algal) toxins from *Microcystis aeruginosa* on isolated enterocytes from the chicken small intestine, *Toxicon*, **30**: 790-793.
63. Falconer, I.R., 1996, Health implications of cyanobacterial (blue-green algal) toxins. In: *Toxic Cyanobacteria: Current Status of Research and Management*, Steffensen, D. A., and Nicholson, B.C [Eds.]. Australian Centre for Water Quality Research, p. 61-65.
64. Fastner, J., Heinze, R., Chorus, I., 1995, Microcystin-content, hepatotoxicity and cytotoxicity of cyanobacteria in some German water bodies, *Water Sci. Technol*., **32**: 165-170.
65. Fastner, J., Codd, G.A., Metcalf, J.S., Woitke, P., Wiedner, C., Utkilen, H., 2002, An international intercomparison exercise for the determination of purified microcystin-LR and microcystins in cyanobacterial field material, *Anal. Bioanal. Chem.*, **374**: 437-444.
66. Fawell, J.K., James, C.P., James, H.A., 1994, *Toxins from Blue-Green Algae: Toxicological Assessment of Microcystin-LR and a Method for its Determination in Wate*r, Foundation for Water Research, Marlow, Bucks, England.
67. Fay, P., Van Baalen, C. [Eds], 1987,*The Cyanobacteria*, Elsevier, Amsterdam.
68. Fischer, W.J., Dietrich, D.R., 2000, Pathological and biochemical characterization of microcystin-induced hepatopancreas and kidney damage in carp (*Cyprinus carpio*), *Toxicol. Appl. Pharmacol*., **64**: 73-81.
69. Fischer, W.J., Tencalla, F., Hitzfeld, B.C., Mikhailov, A., Eriksson, J.E., Dietrich, D.R., 2000, Microcystin-LR toxicodynamics, induced pathology, and immunohistochemical localization in livers of blue-green algae exposed rainbow trout (*Oncorhynchus mykiss*), *Tox. Sci*., **54**: 365-373.
70. Fischer, W.J., Altheimer, S., Cattori, V., Meier, P.J., Dietrich, D.R., Hagenbuch, B., 2005, Organic anion transporting polypeptides expressed in liver and brain mediate uptake of microcystin, *Toxicol. Appl. Pharmacol*., **203**: 257-63.
71. Fladmark, K.E., Serres, M.H., Larsen, N.L., Yasumoto, T., Aune, T., Døskeland, S.O., 1998, Sensitive detection of apoptogenic toxins in suspension cultures of rat and salmon hepatocytes, *Toxicon*, **36**: 1101–1114.

72. Fladmark, K.E., Brustugun, O.T., Mellgren, G., Krakstad, C., Boe, R., Vintermyr, O.K., Schulman, H., Doskeland, S.O., 2002, Ca^{2+}/calmodulin-dependent protein kinase II is required for microcystin-induced apoptosis, *J. Biol. Chem*., **277**: 2804-2811.
73. Francis, G., 1878, Poisonous Australian lakes, *Nature*, **18**: 11-12.
74. Frangez, R., Zuzek, M.C., Mrkun, J., Suput, D., Sedmak, B., Kosec, M., 2003, Microcystin-LR effects cytoskeleton and morphology of rabbit primary whole embryo cultured cells *in vitro*, *Toxicon*, **41**: 999-1005.
75. Fricker, G., Hugentobler, G., Meier, P.J., Kurz, G., Boyer, J.L., 1987, Identification of a single sinusoidal bile salt uptake system in skate liver, *Am. J. Physiol. Gastrointest. Liver Physiol*., **253**: 816–822.
76. Fu, W.Y., Chen, J.P., Wang, X.M., Xu, L.H., 2005, Altered expression of p53, Bcl-2 and Bax induced by microcystin-LR *in vivo* and *in vitro*, *Toxicon*, **46**: 171–177.
77. Fujiki, H., Suganuma, M., 1994, Tumor necrosis factor-alpha, a new tumor promoter, engendered by biochemical studies of okadaic acid, *J. Biochem. (Tokyo)*, **115**: 1-5.
78. Gehringer, M. M., 2004, Microcystin-LR and okadaic acid-induced cellular effects: a dualistic response, *FEBS. Lett*., **557**: 1-8.
79. Goldberg, J., Huang, H.B., Kwon, Y.G., Greengard, P., Nairn, A.C., Kuriyan, J., 1995, Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1, *Nature*, **376**: 745–753.
80. Gulledge, B., Aggen, J.B., Huang, H-B., Nairn, A.C., Chamberlin, A.R., 2002, The microcystins and nodularins: Cyclic Polypeptide Inhibitors of PP1 and PP2A, *Curr. Med. Chem*., **9**: 1991-2003.
81. Gupta, S., 1998, Cyanobacterial toxins: Microcystin-LR. In: Guidelines for drinking water quality, *Health criteria and other supporting information*. World Health Organization: 95-110.
82. Guzman, R.E., Solter, P.F., 2002, Characterization of sublethal microcystin-LR exposure in mice, *Vet. Pathol*., **39**: 17–26.
83. Guzman, R.E., Solter, P.F., Runnegar, M.T., 2003, Inhibition of nuclear protein phosphatase activity in mouse hepatocytes by the cyanobacterial toxin microcystin-LR, *Toxicon*, **41**: 773-781.
84. Hagenbuch, B., Meier, P.J., 2004, Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/functional properties, *Pflugers Arch*., **447**: 653-65.
85. Hamm-Alvarez, S.F., Wei, X., Berndt, N., Runnegar, M., 1996, Protein phosphatases independently regulate vesicle movement and microtubule subpopulations in hepatocytes, *Am. J. Physiol*., **271**: 929-943.
86. Harada, K., Matsuura, K., Suzuki, M., Watanabe, M.F., Oishi, S., Dahlem, A.M., Beasley, V.R., Carmichael, W.W., 1990, Isolation and characterization of the minor components associated with microcystins LR and RR in the cyanobacterium (blue-green algae), *Toxicon*, **28**: 55–64.
87. Hastie, C.J., Borthwick, E.B., Morrison, L.F., Codd, G.A., Cohen, P.T.W., 2005, Inhibition of several protein phosphatases by a non-covalently interacting microcystin and a novel cyanobacterial peptide, nostocyclin, *Biochim. Biophys. Acta*, **1726**: 187-193.
88. Hermansky, S.J., Stohs, S.J., Eldeen, Z.M., Roche, V.F., Mereish, K.A., 1991, Evaluation of potential chemoprotectants against microcystin-LR hepatotoxicity in mice, *J. Appl. Toxicol*., **11**: 65-73.
89. Hitzfeld, B.C., Hoger, S.J., Dietrich, D.R., 2000, Cyanobacterial toxins: removal during drinking water treatment, and human risk assessment, *Environ. Health Perspect*., **108**: 113-122.
90. Holen, I., Gordon, P.B., Seglen, P.O., 1993, Inhibition of hepatocytic autophagy by okadaic acid and other protein phosphatase inhibitors, *Eur. J. Biochem*., **215**: 113-122.
91. Honkanen, R.E., Zwiller, J., Moore, R.E., Daily, S.L., Khatra, B.S., Dukelow, M. Boynton, A.L., 1990, Characterization of microcystin-LR, a potent inhibitor of type 1 and type 2A protein phosphatases, *J. Biol. Chem*., **265**: 19401-19404.
92. Honkanen, R.E, Golden, T., 2002, Regulators of serine/threonine protein phosphatases at the dawn of a clinical era?, *Curr. Med. Chem*., **9**: 2055–2075.
93. Hooser, S.B., 2000, Fulminant hepatocyte apoptosis *in vivo* following microcystin-LR administration to rats, *Toxicol. Pathol*., **28**: 726–733.
94. Humpage, A.R. Falconer, I.R., 1999, Microcystin-LR and liver tumor promotion: Effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes, *Environ. Toxicol.*, **14**: 61-75.
95. Humpage, A.R., Hardy, S.J., Moore, E.J., Froscio, S.M., Falconer, I.R., 2000, *Microcystins* (cyanobacterial toxins) in drinking water enhance the growth of aberrant crypt foci in the mouse colon, *J. Toxicol. Environ. Health A*, **61**: 155-165.
96. Humphrey, J.M., Aggen, J., Chamberlin, A.R., 1996, Synthesis of the serine-threonine phosphatase inhibitor microcystin LA, *J. Am. Chem. Soc*., **118**: 11759-11770.
97. Hunter, T., 1995, Protein kinases and phosphatases: The yin and yang of protein phosphorylation and signalling, *Cell*, **80**: 225-236.

98. Hyde, E.G., Carmichael, W.W., 1991, Anatoxin-*a* (S) a naturally occurring organophosphate, is an irreversible active site-directed inhibitor of acetylcholinesterase (EC 3.1.1.7), *J. Biochem. Toxicol*., **6**: 195-201.
99. Hyenstrand, P., Metcalf, J.S., Beattie, K.A., Codd, G.A., 2001, Losses of the cyanobacterial toxin microcystin-LR fom aqueous solution by absorption during laboratory manipulations, *Toxicon*, **39**: 589-594.
100. Ingebritsen, T.S., Cohen, P., 1983, Protein phosphatases: properties and role in cellular regulation, *Science,* **221**: 331-338.
101. Ito, E., Kondo, E., Terao, K., Harada, K., 1997, Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin-LR, *Toxicon*, **35**, p.1453–1457.
102. Ito, E., Kondo, F., Harada, K., 2000, First report on the distribution of orally administered microcystin-LR in mouse tissue using an immunostaining method, *Toxicon*, **38**, p. 37-48.
103. Ito, E., Takai, A., Kondo, F., Masui, H., Imanishi, S., Harada, K., 2002, Comparison of protein phosphatase inhibitory activity and apparent toxicity of microcystins and related compounds, *Toxicon*, **40**, p. 1017-1025.
104. Jacquet, C., Thermes, V., De Luze, A., Puiseux-Dao, S., Bernard, C., Joly, J.S., Bourrat, F., Edery, M., 2004, Effects of microcystin-LR on development of medaka fish embryos(*Oryzias latipes*), *Toxicon*, **43**, p. 141-7.
105. Jones, G. J., Orr, P. T., 1994, Release and degradation of microcystin following algicide treatment of a *Microcystis aeruginosa* bloom in a recreational lake, as determined by HPLC and protein phosphatases assay, *Water Res*., **28**, p. 871-876.
106. Karp G., 2005, *Cell and Molecular Biology: Concepts and Experiments*, Fourth ed,. John Wiley and Sons, Hoboken, NJ, p. 346-358.
107. Kaya, K., Watanabe, M. M., 1990, Microcystin composition of an axenic clonal strain of *Microcystis viridis* and *Microcystis viridis*-containing waterblooms in Japanese freshwaters, *J. Appl. Phycol*., **2**, p. 173.
108. Keleti, G., Sykora, J. L., 1982, Production and properties of cyanobacterial endotoxins, *Appl. Environ. Microbiol*., **43**, p. 104-109.
109. Kondo, F., Ikai, Y., Oka, H., Ishikawa, N., Watanabe, M. F., Watanabe, M., Harada, K., Suzuki, M., 1992, Separation and identification of microcystins in cyanobacteria by frit-fast atom bombardment liquid chromatography / mass spectrometry, *Toxicon*, **30**, p. 227-237.
110. Kondo, F., Matsumoto, H., Yamada, S., Ishikawa, N., Ito, E., Nagata, S., Ueno, Y., Suzuki, M., Harada, K., 1996, Detection and identification of metabolites of microcystins formed *in vivo* in mouse and rat livers, *Chem. Res. Toxicol*., **9**, p. 1355–1359.
111. Konst, H., McKercher, P. D., Gorham P. R., Robertson A., Howell J., 1965, Symptoms and pathology produced by toxic *Microcystis aeruginosa* NRC-1 in laboratory and domestic animals, *Can. J. Comp. Med. Vet. Sci*., **29**, p. 221-228.
112. Krakstad, C., Herfindal, L., Gjertsen, B. T., Boe, R., Vintermyr, O. K., Fladmark, K. E., Doskeland, S. O., 2005, CaM-kinaseII-dependent commitment to microcystin-induced apoptosis is coupled to cell budding, but not to shrinkage or chromatin hypercondensation, *Cell Death Diff*.,(ahead of print).
113. Kuiper- Goodman, T., Gupta, S., Caombley, H., Thomas, B. H., 1994, Microcystins in drinking water: Risk, assessment and derivation of a possible guidance value for drinking water, In: *Proc.of Toxic Cyanobacteria – A Global Perspective. Australian Center of Water Quality Research*. Australia.
114. Kujbida, P., Hatanaka, E., Campa, A., Colepicolo, P., Pinto, E., 2006, Effects of microcystins on human polymorphonuclear leukocytes, *Biochem. Biophys. Res*. *Commun*., **341**, p. 273-277.
115. Lakshmana Rao, P. V., Gupta, N., Jayaraj, R., 2004, Screening of certain chemoprotectants against cyclic peptide toxin microcystin-LR, *Indian J. Pharmacol*., **36**, p. 87-92.
116. Lankoff, A., Kolataj, A., 2001, Influence of microcystin-YR and nodularin on the activity of some proteolytic enzymes in mouse liver, *Toxicon*, **39**, p. 419-423.
117. Lankoff, A., Carmichael, W. W., Grasman, K. A., Yuan, M., 2004, The uptake kinetics and immunotoxic effects of microcystin-LR in human and chicken peripheral blood lymphocytes *in vitro*, *Toxicology*, **204**, p. 23-40.
118. Lankoff, A., Kolataj, A., 2001, Influence of microcystin-YR and nodularin on the activity of some proteolytic enzymes in mouse liver, *Toxicon*, **39**, p. 419-423.
119. LeClaire, R. D., Parker, G. W., Franz, D. R., 1995, Hemodynamic and calorimetric changes induced by microcystin-LR in the rat, *J. Appl. Toxicol*., **15**, p. 303-311.
120. Li, X. Y., Chung, I. K., Kim, J. I., Lee, J. A., 2004, Subchronic oral toxicity of microcystin in common carp (*Cyprinus carpio* L.) exposed to *Microcystis* under laboratory conditions, *Toxicon*, **44**, p. 821-827.
121. Lovell, R. A., Schaeffer, D. J., Hooser, S. B., Haschek, W. M., Dahlem, A. M., Carmichael, W. W., Beasley, V. R., 1989, Toxicity of intraperitoneal doses of microcystin-LR in two strains of male mice, *J. Env. Pathol. Toxicol. Oncol*., **9**, p. 221–237.

122. Lukac, M., Aererter, R., 1993, Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*, *Toxicon*, **31**, p. 293- 305.
123. MacKintosh, C., Beattie, K. A., Klumpp, S., Cohen, P., Codd, G. A., 1990, Cyanobacterial microcystin- LR is a potent and specific inhibitor of protein phosphatases 1 and 2a from both mammals and higher plants, *FEBS Lett*., **264**, p. 187–192.
124. Madigan, M. T., Martinko, J. M., Parker, J., 1997, *Brock Biology of Microorganisms*, Eighth edition. Prentice Hall.
125. Malbrouck, C., Trausch, G., Devos, P., 2004, Effect of microcystin-LR on protein phosphatase activity in fed and fasted juvenile goldfish *Carassius auratus* L,. *Toxicon*, **43**, p. 295-301.
126. Malbrouck, C., Trausch, G., Devos, P., Kestemont, P. D., 2004, Effect of microcystin-LR on protein phosphatases activity and glycogen content in isolated hepatocytes of fed and fasted juvenile goldfish *Carassius auratus* L, *Toxicon*, **44,** p. 927-932.
127. Mankiewicz, J., Walter, Z., Tarczynska, M., Palyvoda, O., Wojtysiak-Staniaszczyk, M., Zalewski, M., 2002, Genotoxicity of cyanobacterial extracts containing microcystins from Polish water reservoirs as determined by SOS chromotest and comet assay, *Environ. Toxicol*., **17**, p. 341-350.
128. Marahiel, M. A., Nakano, M. M., Zuber, P., 1993, Regulation of peptide antibiotic production in *Bacillus*, *Mol. Microbiol*., **7**, p. 631–636.
129. McDermott, C. M., Nho, C. W., Howard, W., Holton, B., 1998, The cyanobacterial toxin, microcystin-LR, can induce apoptosis in a variety of cell types, *Toxicon*, **36**, p. 1981-1996.
130. McElhiney, J., Lawton, L. A., 2005, Detection of the cyanobacterial hepatotoxins microcystins, *Toxicol. Appl. Pharmacol.*, **203**, p. 219-230.
131. Mereish, K. A., Solow, R., 1990, Effect of antihepatotoxic agents against microcystin-LR toxicity in cultured rat hepatocytes, *Pharm. Res*., **7**, p. 256-259.
132. Mereish, K. A., Bunner, D. L., Ragland, D. R., Creasia, D. A., 1991, Protection against microcystin-LR-induced hepatotoxicity by Silymarin: biochemistry, histopathology, and lethality, *Pharm. Res*., **8**, p. 273-277.
133. Meriluoto, J., Sandstrom, A., Eriksson, J. E., Remaud, G., Craig, A. G., Chattopadhyaya, J., 1989, Structure and toxicity of a peptide hepatotoxin from the cyanobacterium *Oscillatoria agardhii*, *Toxicon*, **27**, p. 1021-1034.
134. Mikalsen, B., Boison, G., Skulberg, O. M., Fastner, J., Davies, W., Gabrielsen, T. M., Rudi, K., Jakobsen, K. S., 2003, Natural variation in the microcystin synthetase operon mcyABC and impact on microcystin production in *Microcystis* strains, *J. Bacteriol.*, **185**, p. 2774-2785.
135. Mikhailov, A., Harmala-Brasken, A. S., Hellman, J., Meriluoto, J., Eriksson, J. E., 2003, Identification of ATP-synthase as a novel intracellular target for microcystin-LR, *Chem. Biol. Interact*., **142**, p. 223-237.
136. Milutinovic, A., Sedmak, B., Horvat-Znidarsic, I., Suput, D., 2002, Renal injuries induced by chronic intoxication with microcystins, *Cell. Mol. Biol. Lett*., **7**, p. 139-141.
137. Milutinovic, A., Zivin, M., Zorc-Pleskovic, R., Sedmak, B., Suput, D., 2003, Nephrotoxic effects of chronic administration of microcystins -LR and -YR, *Toxicon*, **42**, p. 281-288.
138. Miura, G. A., Robinson, N. A., Lawrence, W. B., Pace, J. G., 1991, Hepatotoxicity of microcystin-LR in fed and fasted rats, *Toxicon*, **29**, p. 337-346.
139. Moffitt, M. C., Neilan, B. A., 2004, Characterization of the nodularin synthetase gene cluster and proposed theory of the evolution of cyanobacterial hepatotoxins, *Appl. Environ. Microbiol*., **70**, p. 6353-6362.
140. Moreno, I., Pichardo, S., Jos, A., Gomez-Amores, L., Mate, A., Vazquez, C. M., Camean, A. M., 2005, Antioxidant enzyme activity and lipid peroxidation in liver and kidney of rats exposed to microcystin-LR administered intraperitoneally, *Toxicon*, **45**, p. 395-402.
141. Mumby, M., Walter, G., 1993, Protein serine/threonine phosphatases: structure, regulation and functions in cell growth, *Physiol. Rev*., **73**, p. 673-699.
142. Nakano, M., Nakano, Y., Saito-Taki, T., Mori, N., Kojima, M., Ohtake, A., Shirai, M., 1989, Toxicity of *Microcystis aeruginosa* K-139 strain, *Microbiol. Immunol*., **33**, p. 787-792.
143. Nakano, Y., Shirai, M., Mori, N., Nakano, M., 1991, Neutralization of microcystin shock in mice by tumor necrosis factor alpha antiserum, *Appl. Environ. Microbiol*., **57**, p. 327-330.
144. Naseem, S. M., Hines, H. B., Creasia, D. A., 1990, Inhibition of microcystin-induced release of cyclooxygenase products from rat hepatocytes by anti-inflammatory steroids, *Proc. Soc. Exp. Biol Med*., **195**, p. 345-349.
145. Neilan, B. A., Dittmann, E., Rouhiainen, L., Bass, R. A., Schaub, V., Sivonen, K., Börner, T., 1999, Nonribosomal peptide synthesis and toxigenicity of cyanobacteria, *J. Bacteriol*., **181**, p. 4089-4097.
146. Nishiwaki-Matsushima, R., Ohta, T., Nishiwaki, S., Suganuma, M., Kohyama, K., Ishikawa, T., Carmichael, W. W., Fujiki, H., 1992, Liver tumour promotion by the cyanobacterial cyclic peptide toxin microcystin-LR, *J. Cancer Res. Clin. Oncol.*, **118**, p. 420-424.

147. Nishizawa, T., Asayasma, M., Fujii, K., Harada, K.-I., Shirai, M., 1999, Genetic analysis of the peptide synthetase genes for a cyclic heptapeptide microcystin in *Microcystis spp*, *J. Biochem*., **126**, p. 520-529.
148. Nishizawa, T., Ucda, A., Asayama, M., Fujii, K., Harada, K. I., Ochi, K., Shirai, M., 2000, Polyketide syntase gene coupled to the peptide synthetase module involved in the biosynthesis of the cyclic heptapeptide microcystin, *J. Biochem*., **127**, p. 779-789.
149. Oh, H. M., Lee, S. J., Jang, M. H., Yoon, B. D., 2000, Microcystin production by *Microcystis aeruginosa* in a phosphorus-limited chemostat, *Appl. Environ. Microbiol.*, **66**, p. 176–179.
150. Park, H. D., Iwami, C., Watanabe, M. F., Harada, K. I., Okino, T., Hayashi, H., 1998, Temporal variabilities of the concentrations of intra and extracellular microcystin and toxic *Microcystis* species in a hypertrophic Lake, Lake Suwa, Japan (1991-1994), *Environ. Toxicol. Water Qual*., **13**, p. 61-72.
151. Pearson, M. J., 1990, *Toxic blue-green algae*, A report by the UK National Rivers Authority, Water Quality, **2**, p. 1-128.
152. Pearson, L. A., Hisbergues, M., Borner, T., Dittmann, E., Neilan, B. A., 2004, Inactivation of an ABC transporter gene, mcyH, results in loss of microcystin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806, *Appl. Environ. Microbiol.*, **70**, p. 6370-6378.
153. Pflugmacher, S., Wiegand, C., Oberemm, A., Beattie, K. A., Krause, E., Codd, G. A., Steinberg, C. E., 1998, Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: the first step of detoxication, *Biochim. Biophys. Acta*, **1425**, p. 527-33.
154. Pflugmacher, S., 2002, Possible allelopathic effects of cyanotoxins, with reference to microcystin-LR, in aquatic ecosystems, *Environ. Toxicol*., **17**, p. 407-413.
155. Pichardo, S., Jos, A., Zurita, J. L., Salguero, M., Camean, A.M., Repetto, G., 2005, The use of the fish cell lines RTG-2 and PLHC-1 to compare the toxic effects produced by microcystins LR and RR, *Toxicol. in Vitro*, **19**, p. 865-873.
156. Pichardo, S., Jos, A., Zurita, J., Salguero, M., Camean, A. M., Repetto, G., 2006, Toxic effects produced by microcystins from a natural cyanobacterial bloom and a *Microcystis aeruginosa* isolated strain on the fish cell lines RTG-2 and PLHC-1, *Arch. Environ. Contam. Toxicol*., (Pub ahead of print).
157. Pouria, S., De Andrade, A., Barbarosa, J., Cavalcanti, R. L., Barreto, V. T. S., Ward, O. J., Preiser, W., Poon, G. K., Neild, G. H., Codd, G. A., 1998, Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil, *Lancet*, **352**, p. 21-26.
158. Rabergh, C. M., Ziegler, K., Isomaa, B., Lipsky, M. M., Eriksson, J. E., 1994, Uptake of taurocholic acid in isolated hepatocytes from rainbow trout, *Am. J. Physiol. Gastrointest. Liver Physiol*., **3**, p. 380–386.
159. Rai, A. N., 1990, CRC *Handbook of Symbiotic Cyanobacteria*, CRC Press, Boca Raton.
160. Rami, B. G., Chin, L. S., Lazio, B. E., Singh, S. K., 2003, Okadaic acid induced apoptosis in malignant glioma cells, *Neurosurg. Focus*., **14**, p.1-4.
161. Rantala, A., Fewer, D., Hisbergues, M., Rouhiainen, L., Vaitomaa, J., Böerner, T., Sivonen, K., 2004,Phylogenetic evidence for the early evolution of microcystin synthesis, *PNAS*, **101**, p. 568-573.
162. Rao, P. V. L., Gupta, N., Jayaraj, R., Bhaskar, A. S. B., Jatav, P. C., 2005, Age-dependent effects on biochemical variables and toxicity induced by cyclic peptide toxin microcystin-LR in mice, *Comp. Biochem. Physiol. Part C*., **140**, p. 11-19.
163. Rapala, J., Sivonen, K., Lyra, C., Niemela, S. I., 1997, Variation of microcystins, cyanobacterial hepatotoxins, in *Anabaena spp*. as a function of growth stimuli, *Appl. Environ. Microbiol*., **63**, p. 2206-2212.
164. Repavich, W., Sonzogni, W. C., Standridge, J. H., Wedepohl, R. E., Meisner, L. E., 1990, Cyanobacteria (blue green algae) in Wisconsin waters: acute and chronic toxicity, *Water Res*., **24**, p. 225-231.
165. Rinehart, K. L., Harada, K.-I., Namikoshi, M., Chen, C., Harvis, C. A., Munro, M. H. G., Blunt, J. W., Mulligan, P. E., Beasley, V. R., Dahlem, A. M., 1988, Nodularin, microcystin, and the configuration of Adda, *J. Amer. Chem. Soc*., **110**, p. 8557- 8558.
166. Rinehart, K. L., Namikoshi, M., Choi, B. W., 1994, Structure and biosynthesis of toxins from blue green algae (cyanobacteria), *J. Appl. Phycol*., **6**, p. 159–176.
167. Rohrlack, T., Dittmann, E., Henning, M., Borner, T., Kohl, J.-G., 1999, Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by cyanobacterium *Microcystis aeruginosa*, *Appl. Environ. Microbiol*., **65**, p. 737–739.
168. Rouhiainen, L., Vakkilainen, T., Siember, B. L., Buikema, W., Haselkorn, R., Sivonen, K., 2004, Genes coding for hepatotoxic heptapeptides (microcystins) in the cyanobacterium *Anabaena strain* 90, *Appl. Environ. Microbiol*., **70**, p. 686-692.
169. Runnegar, M. T. C., Andrews, J., Gerdes, R. G., Falconer, I. R., 1987, Injury to hepatocytes induced by a peptide toxin from the cyanobacterium *Microcystis aeruginosa*, *Toxicon*, **25**, p. 1235–1239.
170. Runnegar, M. T., Gerdes, R.G., Falconer, I.R., 1991, The uptake of the cyanobacterial hepatotoxin microcystin by isolated rat hepatocytes, *Toxicon*, **29**, p. 43-51.

171. Runnegar, M. T., Kong, S., Berndt, N., 1993, Protein phosphatase inhibition and *in vivo* hepatotoxicity of microcystins, *Am. J. Physiol*., **265**, p. 224-230.
172. Runnegar, M., Berndt, N., Kong, S. M., Lee, E. Y., Zhang, L., 1995, *In vivo* and *in vitro* binding of microcystin to protein phosphatases 1 and 2A, *Biochem. Biophys. Res. Commun*., **216**, p.162–169.
173. Runnegar, M. T., Wei, X. H., Hamm-Alvarez, S. F., 1999, Increased protein phosphorylation of cytoplasmic dynein results in impaired motor function, *Biochem. J*., **342**, p.1-6.
174. Schopf, J. W.,1991, Collapse of the late Proterozoic ecosystem, *South Afr. J. Geol*., **94**, p. 33-43.
175. Sedmak, B., Kosi, G., 1998, The role of microcystins in heavy cyanobacterial bloom formation, *J. Plankton Res*., **20**, p. 691-708.
176. Sedmak, B., Suput, D., 2002, Co-operative effects in tumorigenicity. The microcystin example, *Radiol. Oncol*., **36**, p. 162-164.
177. Shi, L., Charmichael, W. W., Kennelly, P. J.,1999, Cyanobacterial PPP family proteins possess multifunctional capabilities and are resistant to microcystin-LR, *J. Biol. Chem.*, **274**, p. 10039-10046.
178. Sivonen, K., 1990, Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains, *Appl. Environ. Microbiol.*, **56**, p. 2658–2666.
179. Sivonen, K., 1996, Cyanobacterial toxins and toxin production, *Phycologia*, **35**, p. 12-24.
180. Sivonen, K., Jones, J.,1999, Cyanobacterial toxins. *In Toxic Cyanobacteria in Water: A Guide to Public Health Significance, Monitoring and Management*, London. Spon/Chapman& Hall. P, p. 41-111.
181. Solter, P. F., Wollenberg, G. K., Huang, X., Chu, F. S., Runnegar, M. T., 1998, Prolonged sublethal exposure to the protein phosphatase inhibitor microcystin-LR results in multiple dose-dependent hepatotoxic effects, *Toxicol. Sci*., **44**, p. 87-96.
182. Stappenbeck, T. S., Lamb, J. A., Corcoran, C. M., Green, K. J., 1994, Phosphorylation of the desmoplakin COOH-terminus negatively regulates its interaction with keratin intermediate filament networks, *J. Biol. Chem.*, **269**, p. 29351–29354.
183. Stotts, R. R., Namikoshi, M., Haschek, W. M., Rinehart, K. L., Carmichael, W. W., Dahlem, A. M., Beasley, V. R., 1993, Structure modifications imparting reduced toxicity in microcystins from *Microcystis spp*, *Toxicon*, **31**, p. 783-789.
184. Sugaya, Y., Yasuno, M., Yani, T., 1990, Effects of toxic *Microcystis viridis* and isolated toxins on goldfish, *Jpn. J. Limnol*., **51**, p. 149-153.
185. Takenaka, S., 2001, Covalent glutathione conjugation to cyanobacterial hepatotoxin microcystin LR by F344 rat cytosolic and microsomal glutathione S-transferase, *Environ. Toxicol. Pharmacol*., **9**, p. 135–139.
186. Tencalla, F. G., Dietrich, D. R., Schlatter, C., 1994, Toxicity of *Microcystis aeruginosa* peptide toxin to yearling rainbow trout (*Oncorhyunchus mykiss*), *Aquat. Toxicol.*, **30**, p. 215–224.
187. Tencalla, F., Dietrich, D., 1997, Biochemical characterization of microcystin toxicity in rainbow trout (*Oncorhynchus mykiss*), *Toxicon*, **35**, p. 583–595.
188. Teneva, I., Asparuhova, D., Dzhambazov, B., Mladenov, R., Schirmer, K., 2003, The freshwater cyanobacterium *Lyngbya aerugineo-coerulea* produces compounds toxic to mice and to mammalian and fish cells, *Environ. Toxicol*., **18**, p. 9-20.
189. Thompson, W. L., Pace, J. G., 1992, Substances that protect cultured hepatocytes from the toxic effects of microcystin-LR, *Toxicol. In Vitro*, 6, p. 579–587.
190. Tillett, D., Dittmann, E., Erhard, M., Von Dohren, H., Börner, T., Neilan, B. A., 2000, Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide-polyketide synthetase system, *Chem. Biol*., **7**, p. 753-764.
191. Tillett, D., Parker, D. L., Neilan, B. A., 2001, Detection of toxigenicity by a probe for the microcystin synthetase A gene (mcyA) of the cyanobacterial genus *Microcystis*: comparison of toxicities with 16S rRNA and phycocyanin operon (Phycocyanin Intergenic Spacer) phylogenies. *Appl. Environ. Microbiol*., **67**, p. 2810-2818.
192. Tirona, R. G., Leake, B. F., Wolkoff, A. W., Kim, R. B., 2003, Human organic anion transporting polypeptide-C (SLC21A6) is a major determinant of rifampicin-mediated pregnane X receptor activation, *J. Pharmacol. Exp. Ther*., **304**, p. 223–228.
193. Toerien, D. F., Scott, W. E., Pitout, M. J., 1976, *Microcystis* toxins: isolation, identification, implications, *Water S. Afr*., **2**, p.160-162.
194. Toivola, D. M., Garrod, D., Goldman, R. D., Eriksson, J. E., 1997, Protein phosphatases maintain the organization and structural interactions of hepatic intermediate filaments, *J. Cell Sci*., **110**, p. 23-33.
195. Toivola, D. M., Omary, M. B., Ku, N-O., Peltola, O., Baribault, H., Eriksson, J. E., 1998, Protein phosphatase inhibition in normal and keratin 8/18 assembly-incompetent mouse strains supports a functional role of keratin intermediate filaments in preserving hepatocyte integrity, *Hepatology*, **28**, p.116-128.

196. Tokuda, H., Nishino, H., Shirahashi, H., Murakami, N., Nagatsu, A., Sakakibara, J., 1996, Inhibition of 12-O- tetradecanoylphorbol-13-acetate promoted mouse skin papilloma by digalactosyl diacylglycerols from the fresh water cyanobacterium *Phormidium tenue*, *Cancer Lett.*, **104**, p. 91-95.
197. Tokuda, H., Ohigashi, H., Koshimuzu, K., Ito, Y., 1986, Inhibitory effects of ursolic and oleanolic acid on skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate, *Cancer Lett.*, **33**, p. 279-285.
198. Towner, R. A., Sturgeon, S. A., Hore, K. E., 2002, Assessment of in vivo oxidative lipid metabolism following acute microcystin-LR-induced hepatotoxicity in rats, *Free Radic. Res.*, **36**, p. 63-71.
199. Utkilen, H., Gjølme, N., 1992, Toxin production by *Microcystis aeruginosa* as a function of light in continuous Cultures and its ecological significance, *Appl. Environ. Microbiol.*, **58**, p. 1321-1325.
200. Utkilen, H., Gjolme, N., 1995. Iron-stimulated toxin production in Microcystis aeruginosa. *Appl. Environ. Microbiol*. **61**, p. 797-800.
201. Van der Westhuizen, A. J., Eloff, J. N., 1985, Effects of temperature and light on toxicity and growth of the blue-green alga *Microcystis aeruginosa* [UV-006], *Planta*, **163**, p. 55-59.
202. Van Montfoort, J. E., Hagenbuch, B., Groothuis, G. M. M., Koepsell, H., Meier, P. J., Meijer, D. K. F., 2003, Drug uptake systems in liver and kidney, *Curr. Drug Metab.*, **4**, p. 185–211.
203. Vasconcelos, V. M., 1995, Uptake and depuration of the heptapeptide toxin microcystin-LR in *Mytillus galloprovincialis*, *Aquat. Toxicol*., **32**, p. 227–237.
204. Vavricka, S. R., Van Montfoort, J., Ha, H. R., Meier, P. J., Fattinger, K., 2002, Interactions of rifamycin SV and rifampicin with organic anion uptake systems of human liver, *Hepatology*, **36**, p. 164–172.
205. Velez, P., Sierralta, J., Alcayaga, C., Fonseca, M., Loyola, H., Johns, D. C., Tomaselli, G. F., Marban, E., Suarez-Isla, B. A., 2001, A functional assay for paralytic shellfish toxins that uses recombinant sodium channels, *Toxicon.*, **39** , p. 929–935.
206. Vesterkvist, P. S., Meriluoto, J. A., 2003, Interaction between microcystins of different hydrophobicities and lipid monolayers, *Toxicon*, **41**, p. 349-355.
207. Wallace, K. B., Starkov, A. A., 2000, Mitochondrial targets of drug toxicity, *Annu. Rev. Pharmacol. Toxicol.*, **40**, p. 353–388.
208. Walsby, A. E., 1987, Mechanisms of buoyancy regulation by planktonic cyanobacteria withgas vesicles, In: *The Cyanobacteria*. P. Fay and C. Van Baalen [Eds]. Elsevier, Amsterdam, p. 377-414.
209. Walter, G., Mumby, M., 1993. Protein serine/threonine phosphatases and cell transformation, *Biochim. Biophys. Acta*, **1175**, p. 207-226.
210. Wang, P. J., Chien, M. S., Wu, F. J., Chou, H. N., Lee, S. J., 2005, Inhibition of embryonic development by microcystin-LR in zebrafish, *Danio rerio*, *Toxicon*, **45**, p. 303-308.
211. Watanabe, M. F., Oishi, S., 1985, Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions, *Appl. Environ. Microbiol*., **49**, p. 1342-1344.
212. Watanabe, M. F., Oishi, S., Harda, K., Matsuura, K., Kawai, H., Suzuki, M., 1988, Toxins contained in *Microcystis* species of cyanobacteria (blue-green algae), *Toxicon*, **26**, p. 1017–1025.
213. Wickstrom, M. L., Khan, S. A., Haschek, W. M., Wyman, J. F., Eriksson, J. E., Schaeffer, D. J., Beasley, V. R., 1995, Alterations in microtubules, intermediate filaments, and microfilaments induced by microcystin-Lr in cultured-cells, *Toxicol. Pathol*., **23**, p. 326-337.
214. Wisseman, A., 2006, p53 protein or BID protein select the route to either apoptosis (programmed cell death) or to cell cycle arrest opposing carcinogenesis after DNA damage by ROS, *Med. Hypoth.*, Pub. Ahead of print.
215. Yoshida, T., Makita, Y., Tsutsumi, T., Nagata, S., Tashiro, F., Yoshida, F., Sekijima, M., Tamura, S.-I., Harada, T., Maita, K., Ueno, Y., 1998, Immunohistochemical localization of microcystin-LR in the liver of mice: a study on the pathogenesis of microcystin-LR-induced hepatotoxicity, *Toxicol. Pathol*., **26**, p. 411–418.
216. Yu, S. Z., 1995, Primary prevention of hepatocellular carcinoma, *J. Gastroenterol. Hepatol*., **10**, p. 674–682.
217. Zegura, B., Sedmak, B., Filipic, M., 2003, Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2, *Toxicon*, **41**, p. 41-48.
218. Zhan, L., Sakamoto, H., Sakuraba, M., Wu, D. S., Zhang, L. S., Suzuke, T., Honma, M., 2004, Genotoxicity of microcystin-LR in human lymphoblastoid TK6 cells, *Mutat Res.*, **557**, p.1-6.
219. Zhu, Y., Zhong, X., Zheng, S., Ge, Z., Du, Q., Suzhang, Z., 2005, Transformation of immortalized colorectal crypt cells by microcystin involving constitutive activation of Akt and MAPK cascade, *Carcinogenesis*, **26**, p. 1207-1214.

MICROCISTINELE: TOXINE CIANOBACTERIENE PUTERNICE

(Rezumat)

Prezența în apele eutrofe a cianobacteriilor capabile să producă un spectru larg de toxine (cianotoxine) a fost semnalată în toate zonele geografice. În funcție de efectele produse, cianotoxinele au fost clasificate ca neurotoxine (anatoxine), dermatotoxine (lingbiatoxine) şi hepatotoxine (microcistine). Dintre toate cianotoxinele, cele mai comune în mediul acvatic sunt microcistinele, responsabile pentru numeroase cazuri de otrăviri şi decese ale animalelor şi chiar ale oamenilor. Eutrofizarea apelor continentale, asociată dezvoltării economice, a cauzat o frecvență din ce în ce mai mare a înfloririlor cianobacteriene toxice ceea ce atras după sine creşterea riscului de expunere la microcistine. Scopul acestei sinteze este de a oferi un scurt sumar al cianobacteriilor capabile să genereze înfloriri şi să sintetizeze cianotoxine. Lucrarea vizează în special microcistinele şi oferă informații începând cu calea de biosinteză a acestor toxine şi terminând cu efectele lor toxice.

Microcistinele – denumite astfel deoarece sunt generate în primul rând, dar nu numai, de specii din genul *Microcystis*, prima microcistină fiind caracterizată la *M. aeruginosa* – sunt heptapeptide monociclice sintetizate în numeroase variante structurale. Toate variantele structurale ale microcistinelor inhibă protein-fosfatazele, în special pe cele hepatice. Capacitatea de biosinteză a microcistinelor a fost evidențiată la mai multe cianobacterii din genurile *Microcystis*, *Anabaena*, *Oscillatoria*, *Planktothrix*, *Nostoc* şi *Anabaenopsis*, atât *in situ* cât şi în culturi de laborator. Microcistinele sunt sintetizate neribosomal de către un complex enzimatic în care rolul principal revine poliketid-sintetazei şi peptid-sintetazei. Complexul multienzimatic este codificat de genele clusterului *mcy* (de aprox. 55 kb) compus – la *Microcystis* – din 2 operoni (*mcy A-C* şi *mcy D-J*).

Microcistinele acționează în mod selectiv asupra țesutului hepatic. Odată ce sunt preluate de hapatocite, ele afectează structura şi funcțiile celulare provocând stres oxidativ şi peroxidarea lipidelor, fragmentarea ADN, dezorganizarea citoscheletului, a organitelor şi a membranei plasmatice precum şi apoptoză/necroză. Moartea survine ca urmare a şocului hemoragic. DL_{50} variază în funcție de varianta structurală (50 µg/kg corp, pentru MCYST-LR, cea mai bine studiată dintre microcistine). Expunerea la doze subletale creşte incidența cancerelor. Activitatea selectivă a microcistinelor asupra hepatocitelor este datorată sistemului de transport al sărurilor biliare, exprimat şi localizat în membrana bazolateră a hepatocitelor (transportorul OATP – „organic anion-transporting polypeptides"), responsabil de preluarea în celule a microcistinelor.

Studiile *in vitro* şi *in vivo* au indicat inhibarea protein-fosfatazelor citoplasmice PP 1 şi PP 2A din celulele animale, fără a inhiba însă protein-fosfatazele din celulele cianobacteriene. Interacțiunea microcistinelor cu protein-fosfatazele afectează (doze subletale) proliferarea celulară şi echilibrul dintre acest proces şi moartea celulară. Studii recente au demonstrat că MCYST-LR induce procese apoptotice în care sunt implicate proteinele BAX-BCL2-BID şi mitocondriile. Cu toate progresele recente obținute în investigarea microcistinelor, în lucrare sunt evidențiate numeroase probleme care sunt încă necunoscute sau insuficient documentate şi sunt identificate pe această bază câteva direcții profitabile pentru cercetările ulterioare.