Effects of Microcin SF608 and Microcystin-LR, Two Cyanotobacterial Compounds Produced by *Microcystis* sp., on Aquatic Organisms

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ABSTRACT: Effects of two cyanobacterial compounds, microcin SF608 and microcystin-LR, were investigated on different physiological parameters of two organisms, the water moss, *Vesicularia dubyana,* and the waterflea, *Daphnia magna.* Both compounds are produced by *Microcystis* species. Microcystin-LR is a potent inhibitor of protein phosphatases 1 and 2A, and microcin SF608 inhibits serine proteases. Other effects of microcystin-LR are well documented in the literature, but adverse effects of microcin SF608 have not been investigated as yet. This study compared the effects of both compounds on detoxication enzymes, microsomal and soluble glutathione-S-transferase (m-, sGST); oxygen stress enzymes, glutathione peroxidase (GP-X), and peroxidase (POD); photosynthetic oxygen production and chlorophyll *a*:chlorophyll *b* ratio. mGST was inhibited by both compounds in both organisms, significantly by microcin SF608, possibly indirectly by inhibition of that serine protease transforming the mGST to its active form. The sGST of *D. magna* was inhibited by microcin SF608, but elevated by microcystin-LR, and elevated by both compounds in *V. dubyana.* The GP-X in *D. magna* was not altered by microcin SF608, but *elevated parallel* to the sGST, whereas the POD in *V. dubyana* was decreased by both. Photosynthetic oxygen production as well as the chlorophyll *a/b* ratio showed typical stress reactions, a decrease of oxygen production, and an increase of chlorophyll *b,* caused both by microcin SF608 and by microcystin-LR. Microcin SF608 was not likely to be detoxified via conjugation to glutathione. The effects of microcin SF608 and microcystin-LR demonstrate that the impact of cyanobacteria on other organisms may not only be directly related to the presently known toxins. © 2002 Wiley Periodicals, Inc. Environ Toxicol 17: 400–406, 2002; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.10065

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INTRODUCTION

The capability of cyanobacteria to produce a range of toxins led to the recognition that they are an increasing problem in

many freshwater ecosystems. The main group of toxins produced by cyanobacteria are the microcystins with more than 60 structure variants, of which the microcystin-LR congener is one of the most toxic (Carmichael, 1994; Watanabe et al., 1996; Codd et al., 1997). Microcystins are cyclic heptapeptides containing D-amino acids and the unusual amino acid Adda. Two of the amino acids are highly variable, and two are less altered, leading to this structural variety. The main biochemical mechanism of their toxicity is the binding and inhibiting of protein phosphatases 1 and 2A, a central control enzyme for metabolism, e.g., the cytoskeleton (MacKintosh et al., 1990).

Because of the high expression of a carrier mechanism in the vertebrate liver, the OATP (unspecific organic anion transporter, bile acid carrier; e.g., Runnegar et al., 1991), these toxins accumulate there, leading to changing liver enzymes, liver necrosis, and sometimes deadly intrahepatic bleeding (Falconer et al., 1983; Erikson et al., 1990; Råberg et al., 1991). Intoxication has been described since the end of the 19th century, ranging from death of cattle after drinking from lakes contaminated with cyanobacteria (Francis, 1878) to a disastrous accident in a dialysis station, where more than 60 patients died (Pouira, 1998).

Up to a certain concentration, the microcystins are metabolizable by organisms via the glutathione S-transferases (Kondo, 1996; Pflugmacher et al., 1998, 2001; Takenaka, 2001). Glutathione S-transferase (GST) is member of the phase II detoxication enzymes, conjugating electrophile substances to glutathione (GSH), thereby increasing their water solubility to support their excretion. A broad substrate specificity is attained by several soluble GST isoenzymes and by a microsomal enzyme. Microcystins are conjugated to GSH through the terminal methylene of N-methyldehydroalanine (Mdha; Kondo, 1992), which is the moiety that binds to the Cys 273 in the active center of PPA1 (MacKintosh, 1995). In addition to the transformation of the microcystins to a more excretable form, the microcystin-SG conjugate is not able to bind covalently to the PPA. Despite this detoxication pathway the microcystins, particularly the LR congener, express their toxicity and cyanobacterial crude extracts are even more potent than the pure microcystins themselves (e.g., Oberemm et al., 1997; Pietsch et al,. 2001). Either the detoxication capacities of the organisms are not sufficient, or other compounds that accompany the microcystins in the cyanobacteria are contributing to the toxicity by their own toxicity or by interference with the detoxication process.

During an ongoing project to characterize metabolites of bloom-forming cyanobacteria, three serine-protease inhibitors were isolated and their structure determined by NMR and MS techniques (Banker and Carmeli, 1999). One of the compounds, microcin SF608, was chosen for the study described below. Microcin SF608 is a linear peptide consisting of two amino acids (L-phenylalanine, 2-carboxy-6-hydroxyoctahydroindole); an α-hydroxy acid, p-hydroxy-

phenyl-lactic acid; and an agmatine (decarboxylated arginine). It inhibits trypsin and has an IC_{50} of 0.5 μg/mL (Banker and Carmeli, 1999). Via this mechanism, it could indirectly interfere with several other cell processes, for example, activation of proenzymes by the proteolytic processes of serine proteases. It also is not known if other, more general functions are affected by microcin SF608, especially, if it acts against plants. The aim of this study was to compare the effects of two compounds produced by *Microcystis* sp., the well-investigated hepatotoxin microcystin-LR and the recently characterized serine-protease inhibitor microcin SF608, on different physiological parameters of two organisms of the aquatic ecosystem. For the study *Vesicularia dubyana,* a water moss, and the invertebrate *Daphnia magna* were chosen as test organisms. The parameters microsomal and soluble glutathione S-transferases (m-, sGST) as indicators of the activity of the detoxication system were measured in both organisms. As enzymes dealing with oxidative stress, activity of glutathione-peroxidase (GP-X) was determined in *D. magna* and of peroxidase (POD) in *V. dubyana.* In the plant photosynthetic oxygen production as well as chlorophyll *a* and *b* content also were quantified as more general metabolic functions that could be altered by this toxic stress.

MATERIALS AND METHODS

Cyanobacterial Compounds

Microcin SF608 was isolated by Banker and Carmeli (1999) from a *Microcystis* sp. bloom by HPLC and characterized by FAB MS and 1-D and 2-D NMR. For the exposures Microcin SF608 was used in concentrations of 0.5, 5.0, 50.0, and 500.0 μg/L. Microcystin-LR was obtained from Professor G. A. Codd (University of Dundee, Scotland, UK). The toxin was produced by the nonaxenic culture of *Microcystis aeruginosa* (PCC 7813) and purified by HPLC according to the procedure explained in Lawton et al. (1995). To compare dose effects of both compounds, microcystin-LR was used in concentrations of 0.5, 5.0, and 50.0 μg/L.

Rearing of the Test Organisms, *Daphnia magna* and *Vesicularia Dubyana*

Both organisms were maintained in room conditions of a temperature of 20°C and a light/dark regime of 14 h light to 10 h dark. Tank water was reverse osmosis water containing $NaHCO_3$ (0.103 g/L), $CaCl_2$ (0.20 g/L), and sea salt (0.10 g/L). *Daphnia magna* were kept in culture for several weeks prior to exposure in 20-L tanks. They were fed with Microcell® algae powder, and 50% of the water was exchanged every 3–5 days. *Vesicularia dubyana* were cultivated for several months, with nutrients supplied by Povasoli's medium (ESI_{SI} 15 mL/L) and an exchange of 50% of the water weekly.
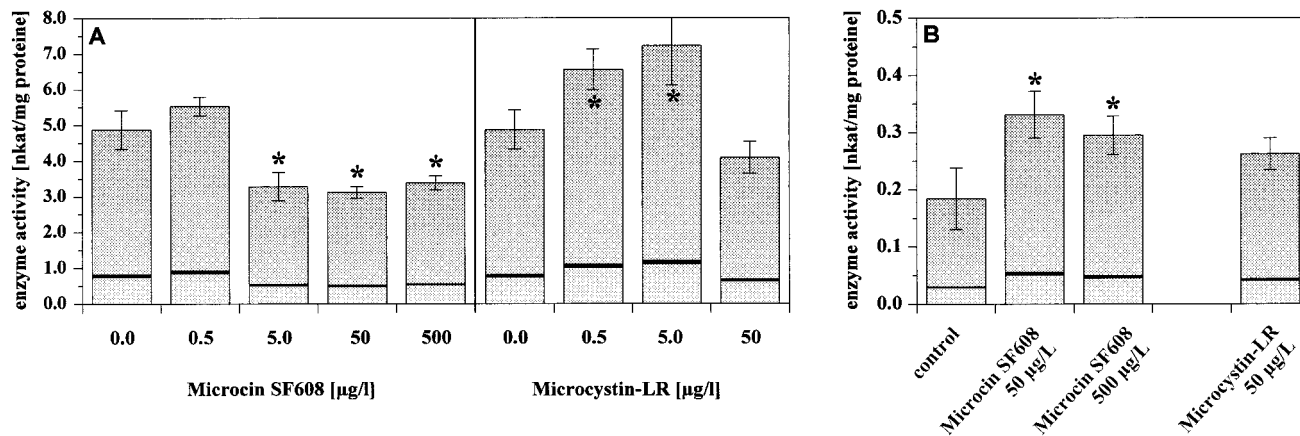
Fig. 1. Activities of soluble glutathione-S-transferase (sGST) in (a) *Daphnia magna* and (b) *Vesicularia dubyana* after exposure to microcin SF608 and microcystin-LR. *Significant changes compared with control values by Newmanns–Keuls test at *p = 0.05.*

Exposure of the Organisms

The organisms were exposed for 24 h without feeding or nutrient supply. Samples for the enzyme measurement were immediately frozen in liquid nitrogen, and stored at −80°C.

Enzyme Preparation and Measurement

Enzymes were extracted from the daphnids according to Wiegand et al. (2000) and from the water moss according to Pflugmacher et al. (1999a). Briefly, the animals were homogenized in sodium phosphate buffer (0.1 M, pH of 6.5, 20% glycerol, 1.4 mM of DTE, 1 mM of EDTA), and the plants ground in liquid nitrogen to a fine powder before suspending in the same buffer. Cell debris was removed by centrifugation. Microsomal and cytosolic enzymes were separated by centrifugation at 106 000 g followed by ammonium sulfate precipitation and desalting. Enzymes were stored in a sodium phosphate buffer (20 mM, pH of 7.0, 1.4 mM of DTE) at −80°C until the measurement of activities.

Enzyme activities were measured colorimetrically: the mGST and sGST according to Habig et al. (1974), using CDNB (1-chloro-2,4-dinitrobenzene) as the photometer substrate; the GP-X according to Drotar et al. (1985), using glutathione reductase for the indirect measurement and NADPH and H_2O_2 as substrate and cosubstrate; and the POD according to Bergmeyer (1983, 1986), using guajacol and H_2O_2 as substrate and cosubstrate. Protein content of the samples was quantified according to Bradford (1974).

Measurement of Photosynthetic Oxygen Production

After exposure, plants were rinsed twice with water and from 5 replicates of 0.5 g of plant material from each toxin exposure, photosynthesis were measured according to

Pflugmacher et al. (1999b), using a Phosy-Mess 400X (Innoconcept GmbH, Berlin, Germany). Oxygen content was determined by a Clarc electrode (WTW EO 196-1.5) and calculated in micromoles of O_2/h*g FW. Measurement conditions were 2000 lx, with a dark/light/dark cycle of 10/12/10 min and a constant temperature of 20°C.

Analysis of Chlorophyll *a* and *b*

Chlorophyll *a* and chlorophyll *b* were extracted in DMSO for 48 h at 8°C according to Inskeep and Bloom (1985). Measurement of the pigments were done colorimetrically at 645 nm and 664.5 nm against DMSO.

RESULTS

Activities of Detoxication Enzymes—Glutathione S-Transferases

In *D. magna,* the sGST was not affected by a microcin SF608 concentration of 0.5 μg/L, but every higher concentration caused a significant inhibition [Fig. 1(a)]. Contrastingly microcystin-LR caused an elevation of activities up to a concentration of 5.0 μg/L; only the highest concentration, 50.0 μg/L, gave no significant change. The mGST was inhibited by microcin SF608 proportional to increasing concentrations, whereas the reaction to microcystin-LR was equivocal [Fig. 2(a)]. The sGST of *V. dubyana* was increased by both cyanobacterial compounds in the concentrations used, significantly with microcin SF608 [Fig. 1(b)], whereas mGST was slightly, but not significantly inhibited [Fig. 2(b)].
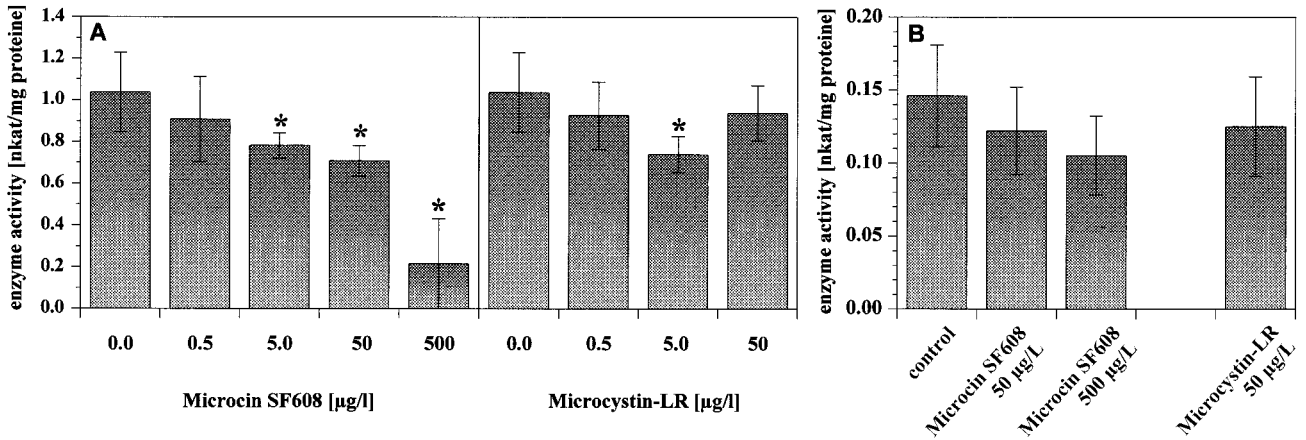
Fig. 2. Activities of microsomal glutathione-S-transferase (mGST) in (a) *Daphnia magna* and (b) *Vesicularia dubyana* after exposure to microcin SF608 and microcystin-LR. *Significant changes compared with control values by Newmanns–Keuls test at *p = 0.05.*

Oxidative Stress Enzymes Peroxidase and Glutathione Peroxidase

The GP-X of *D. magna* showed no definite reaction toward microcin SF608, but exposure to microcystin-LR caused a clear dose-dependent elevation of activities up to 5 μg/L, and a decrease of activity at 50 μg/L [Fig. 3(a)]. The POD of *V. dubyana* was strongly inhibited by microcystin-LR at 50 μg/L, but not by microcin SF608 at the same concentration. The high concentration of microcin SF608 (500 μg/L) caused a nearly total inhibition [Fig. 3(b)].

Photosynthetic Oxygen Production and Pigment Pattern

Photosynthetic oxygen production was strongly affected by both cyanobacterial compounds with a decrease of more

than 50% by both at 50 μg/L and a decrease of more than 75% by microcin SF608 at 500 μg/L [Fig. 4(a)].

The pigment pattern showed the typical stress reaction of plants: chlorophyll *b* values increased by almost a factor of 4 in both compounds in the concentrations used. In addition, the chlorophyll *a* content was nonsignificantly decreased by microcystin-LR and significantly decreased by microcin SF608 [Fig. 4(b)].

DISCUSSION

Both cyanobacterial compounds adversely affected the tested physiological parameters of the organisms. The soluble GSTs of both organisms were increased by microcystin-LR, whereas the microsomal GSTs did not respond so
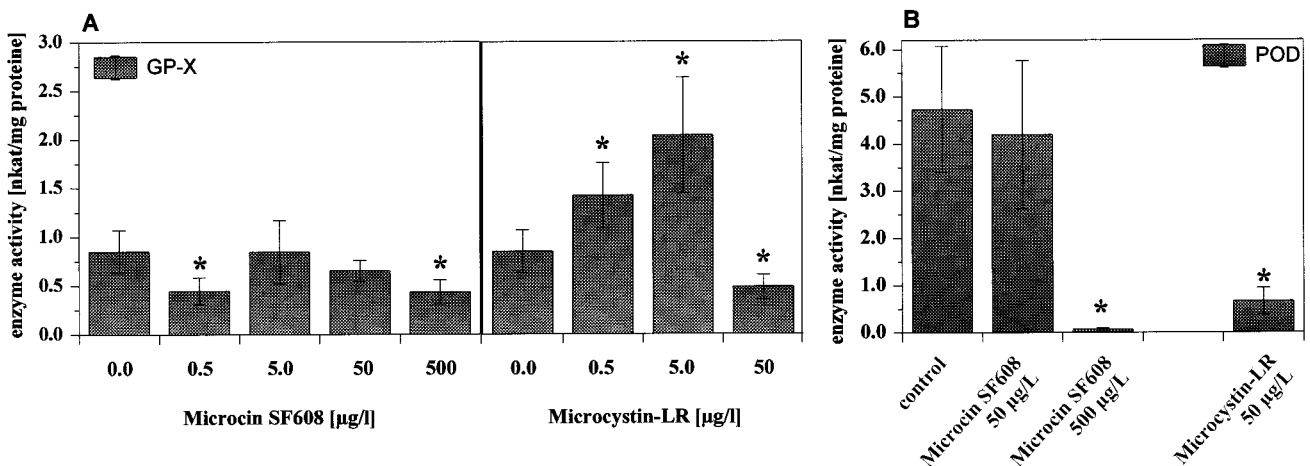

Fig. 3. Activities of (a) glutathione peroxidase (GP-X) in *Daphnia magna* and (b) peroxidase (POD) in *Vesicularia dubyana* after exposure to microcin SF608 and microcystin-LR. *Significant changes compared with control values by Newmanns–Keuls test at *p = 0.05.*
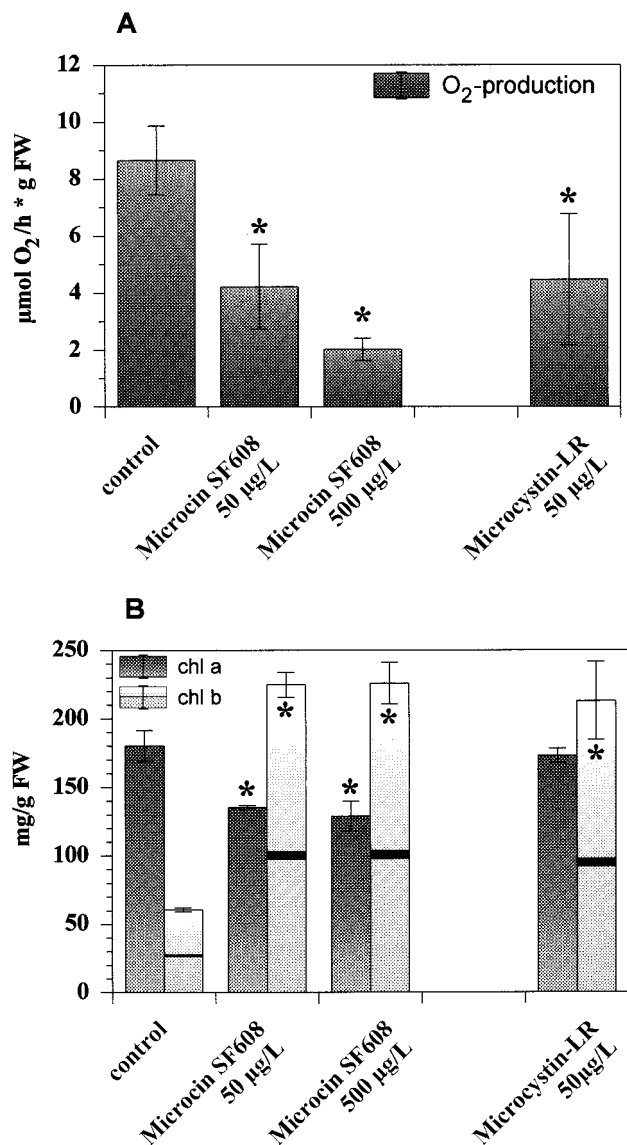
A

B

Fig. 4. (a) Photosynthetic oxygen production and (b) chlorophyll *a* and *b* contents of *Vesicularia dubyana* after exposure to microcin SF608 and microcystin-LR. *Significant changes compared with control values by Newmanns–Keuls test at *p = 0.05*.

clearly. This different reactions of the GSTs toward microcystins was also shown for other organisms, such as the zebrafish, *Danio rerio* (Wiegand, 1999, 2001), the coon tail, *Ceratophyllum demersum* (Pflugmacher et al., 1999c), and the zebra mussel, *Dreissena polymorpha* (Schwalbe and Pflugmacher, personal communication). It is possible that the different GST specificities led to the different reactions or that the microcystins in the cells were distributed more toward the cytosol than toward the membranes.

Microcin SF608 affected the mGST more potently than the sGST—it inhibited the mGST of both organisms but only the sGST of the daphnids. It is possible that this was a result of an indirect mechanism. One major *in vivo* mechanism for activating already synthesized mGST is via limited proteolysis (Morgenstern and Depierre, 1988). In studies by Banker and Carmeli (1999), microcin SF608 showed inhibitory capability toward serin-protease trypsin. An increase of mGST activity is impossible through this mechanism, even if an induction of the enzyme occurred, because the enzyme stays in the inactive form.

sGST consists of several isoenzymes. In *Daphnia magna* seven sGST isoenzymes were isolated (LeBlanc et al., 1988). At a microcin SF608 concentration of 5–500 μg/L, inhibition was detectable, but it was not concentration dependent. Presumably, only distinct isoenzymes were inhibited by this compound, whereas the others were still active. A small amount of microcin SF608 (0.5 μg/L) was not sufficient for inhibition. In the water moss, *Vesicularia dubyana,* activation of sGST was not inhibited by microcin SF608, as activity of sGST actually increased.

Prevention of the transformation of the GST into its active form limits the capacity of the detoxication pathway via the conjugation to glutathione. By this mechanism possible toxicity of microcin SF608 might be explained by the inhibition of the serin proteases, which then cause the inhibition of an important detoxication system in daphnids. From our results it seems very unlikely that microcin SF608 was detoxified via conjugation to glutathione.

The GP-X and POD belong to the group of enzymes dealing with oxidative stress caused by oxygen itself or by reactive oxygen species (ROS). They prevent oxidative damage to the cell, such as lipid peroxidation and DNA or enzyme oxidation. Microcin SF608 did not provoke reactions from GP-X in the daphnids. Contrasted to that, elevation of GP-X by microcystin-LR was concentration dependent, similar to sGST. A parallel elevation of GP-X showed zebra fish embryos after exposure to microcystin-LR (Wiegand et al., 1999). At a microcystin-LR concentration of 50 μg/L the capacity of the enzyme was depleted. There is no evidence about whether the microcystin-LR molecule itself represents or generates reactive oxygen species. The increase in enzyme activity may be caused by simultaneously induction of both GP-X and sGST. The sGST is induced via the DRE (dioxin responsible element) as well as via the ARE (antioxidance responsible element); the latter also induces GP-X (Fernandes et al., 1996). Both compounds caused a decrease in activity of POD in *V. dubyana* but only at high and rarely relevant doses: 500 μ/L microcin SF608 and 50 μg/L microcystin-LR. Further studies are needed to show whether direct binding of the molecules to the enzyme causes this inhibition.

Photosynthesis in *Vesicularia dubyana* was inhibited by both microcin SF608 and microcystin-LR. It has been shown that microcystins inhibit photosynthesis in various plants (Pflugmacher et al., 2001b). Other cyanobacterial compounds such as fisherellin also caused a decrease in the photosynthetic capacity (Srivatava et al., 1998). The stron-

gest inhibition was caused by a cyanobacterial crude extract (Pietsch et al., 2001). Increase of the chlorophyll *b* content with a concurrent decrease in chlorophyll *a* follows a disturbed photosynthetic apparatus. Decrease of the chlorophyll *a* to *b* ratio occurs when the collected light energy cannot be used adequately. This stress reaction of the plant can also be provoked by xenobiotics. Our results, using microcin SF608 and microcystin-LR separately, showed that both were affecting the central energy production mechanism of the plant. The question still remains open whether cyanobacterial secondary metabolites are produced to create an ecologically competitive benefit for the cyanobacteria themselves. That is, as allelochemicals.

It has been shown in several studies that cyanobacterial crude extracts cause different effects than purified toxins or even the equivalent toxin content of the particular crude extract (Fastner et al., 1995; Bury et al., 1996; Oberemm et al., 1997; Pietsch et al., 2001). These results for microcin SF608 and microcystin-LR demonstrate that the effects of cyanobacteria are not related only to the toxins known to date. In the ongoing process of purification and characterization of cyanobacterial compounds, it is likely that more biologically active substances will be revealed. Investigations from different starting points are needed in the ecotoxicological field as well as in the microbial arena to elucidate step by step the natural function of these substances.

REFERENCES

Banker R, Carmeli S. 1999. Inhibitors of serine proteases from a waterbloom of the cyanobacterium *Microcystis* sp. Tetrahedron 55:10835–10844.

Bergmeyer HU, editor. 1983–86. Methods of enzymatic analysis. Volumes 1–12. VCH Weinheim. p 648–653.

Bradford M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal Biochem 72:248–254.

Bury NR, Flik G, Eddy FB, Codd GA. 1996. The effects of cyanobacteria and the cyanobacterial toxin microcystin-LR on Ca^{2+} transport and Na^+/Ka^+-ATPase in *Tilapia* gills. J Exp Biol 199:1319–1326.

Carmichael WW. 1994. The toxins of cyanobacteria. Sci Am 270:78–86.

Codd GA, Ward CJ, Bell SG. 1997. Cyanobacterial toxins: Occurrence, modes of action, health effects and exposure routes. Archiv Toxicol 19:399–410.

Drotar A, Phelps A, Fall R. 1985. Evidence for glutathione peroxidase activities in cultured plant cells. Plant Sci 42:35–40.

Erikson JE, Toivola D, Meriluoto JA, Karaki H, Han YG, Hartshorne D. 1990. Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases. Biochem Biophys Res Com 173:1347–1353.

Falconer I, Beresford AM, Runnegar MTC. 1983. Evidence of liver damage by toxins from a bloom of the blue green algae *Microcystis aeruginosa*. Med J Aust 1:511–514.

Fastner J, Heinze R, Chorus I. 1995. Microcystin-content, hepatotoxicity and cytotoxicity of cyanobacteria in some German water bodies. Water Sci Technol 32(4):165–170.

Fernandes CL, Chavan SJ, Dong JH, Bornmann WG, Polski B, Chisari FV, Montali JA, Schmidi DE, Prochaska HJ. 1996. Regulation of glutathione S-transferases: Clues from a worm, a virus and a mouse with hepatitis. In: Vermeulen NPE, Mulder GJ, Nieuwenhuyse H, Peters WHM, van Bladeren PJ, editors. Glutathione S-transferases, structure, function and clinical implications. London: Taylor & Francis. p 97–109.

Francis G. 1878. Poisonous Australian lake. Nature 18:11–12.

Habig W, Pabst MJ, Jacoby WB. 1974. Glutathione S-transferase: The first step in mercapturic acid formation. J Biol Chem 249:1730–1739.

Inskeep WP, Bloom PR. 1985. Extinction coefficient of chlorophyll *a* and *b* in *N,N*-dimethylformamide and 80% acetone. Plant Physiol 77:483–485.

Kondo F, Ikai Y, Oka H, Okumura M, Ishikawa N, Harada KI, Matsuura K, Murata H, Suzuki M. 1992. Formation, characterization and toxicity of the glutathione and cysteine conjugates of the toxic heptapeptide *Microcystins*. Chem Res Toxicol 5:591–596.

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 9:1355–1359.

Lawton L, Edwards C, Beattie K, Pleasance S, Dear GJ, Codd GA. 1995. Extraction and high performance liquid chromatographic method for the determination of microcystins in raw waters. Nat Toxins 3:50–57.

LeBlanc GA, Hilgenberg B, Cochrane BJ. 1988. Relationships between the structures of chlorinated phenols, their toxicity and their ability to induce glutathione S-transferase activity in *Daphnia magna*. Aquatic Toxicol 12:147–156.

MacKintosh C, Beattie K, Klumpp S, Cohen P, Codd GA. 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Letters 264:187–192.

MacKintosh RW, Dalby KN, Campbell DG, Cohen PTW, Cohen P, MacKintosh C. 1995. The cyanobacterial toxin microcystin binds covalently to cysteine-273 on protein phosphatase 1. FEBS Letters 371:236–240.

Morgenstern R, DePierre JW, Jörnvall H. 1985. Microsomal glutathione transferase, primary structure. J Biol Chem 260:13976–13983.

Oberemm A, Fastner J, Steinberg CEW. 1997. Effects of microcystin-LR and cyanobacterial crude extracts on embryo-larval development of zebrafish (*Danio rerio*). Water Res 31:2918–2921.

Pflugmacher S, Wiegand C, Oberemm A, Beattie KA, Krause E, Codd GA, Steinberg CEW. 1998. Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hep-

atotoxin microcystin-LR: The first step of detoxication. Biochim Biophys Acta 1425:527–533.

Pflugmacher S, Geißler K, Steinberg CEW. 1999a. Activity of phase I and phase II detoxication enzymes in different cormus parts of *Phragmites australis.* Ecotoxicol Environmental Safety 42:62–66.

Pflugmacher S, Spangenberg M, Steinberg CEW. 1999b. Dissolved organic matter (DOM) and effects on the aquatic macrophyte *Ceratophyllum demersum* in relation to photosythesis, pigment pattern and activity of detoxication enzymes. J Appl Bot 73:184–190.

Pflugmacher S, Codd GA, Steinberg CEW. 1999c. Effects of the cyanobacterial toxin microcystin-LR on detoxication enzymes in aquatic plants. Environ Toxicol 14(1):111–115.

Pflugmacher S, Wiegand C, Beattie KA, Krause E, Steinberg CEW, Codd GA. 2001a. Uptake, effects, and metabolism of cyanobacterial toxins in the emergent reed plant *Phragmites australis* (cav.) trin. ex steud. Environ Toxicol Chem 20:846–852.

Pflugmacher S, Amé V, Wiegand C, Steinberg CEW. 2001b. Cyanobacterial toxins and endotoxins—their origin and their ecophysiolocical effects in aquatic organisms. Wasser & Boden, 53/4:15–20.

Pietsch C, Wiegand C, Ame MV, Nicklisch A, Wunderlin D, Pflugmacher S. 2001. The effects of a cyanobacterial crude extract on different aquatic organisms: Evidence for cyanobacterial toxin modulating factors. Environ Toxicol 16:535–542.

Pouria S, De Andrade A, Barbosa J, Cavalcanti RL, Borreto VTS, Ward CJ, Preiser W, Poon-Grace K, Neild GH, Codd GA. 1998. Fatal microcystin intoxications in haemodialysis unit in Carnaru, Brazil. Lancet 253:21–26.

Råberg CMI, Bylund G, Eriksson JE. 1991. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa* on common carp (*Cyprinus carpio*). Aquat Toxicol 20:131–146.

Runnegar MTC, Gerdes RG, Falconer IR. 1991. The uptake of the cyanobacterial hepatotoxin microcystin by isolated rat hepatocytes. Toxicon 29:43–51.

Salin M. 1987. Toxic oxygen species and protective systems of the chloroplast. Physiol Plant 72:681–689.

Schwalbe H, Pflugmacher S. 2000. Personal communication, Leibniz-Institue of Freshwater Biology and Inland Fisheries, Berlin, Germany.

Srivatava A, Jüttner F, Strasser RJ. 1998. Action of the allelochemical, fischerelin A, on photosystem II. Biochim Biophys Acta 1364:326–336.

Takenaka S. 2001. Covalent glutathione conjugation to cyanobacterial hepatotoxin microcystin LR by F344 rat cytosolic and microsomal glutathione S-transferases. Environ Toxicol Phamacol 9(4):135–139.

Watanabe MF, Harada KI, Carmichael WW, Fujiki H. 1996. Toxic *Microcystis.* Boca Raton, FL: CRC Press. p 262 ff.

Wiegand C, Pflugmacher S, Oberemm A, Steinberg CEW. 2000. Activity development of selected detoxication enzymes during the ontogenesis of the Zebrafish (*Danio rerio*). International Reviews of Hydrobiology 85:413–422.

Wiegand C. 2001. Vergleichende Teratogenität, Detoxierung und Metabolismus von Atrazin, Trifluoressigsäure und Microcystin-LR in Embryonalstadien des Zebrabärblings, (*Danio rerio*) [dissertation]. Humboldt University, Berlin, Germany.