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Autor: Grundler, Verena / Gademann, Karl

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Toxins from Cyanobacteria – Chemical and Biological Studies Addressing their Mode of Action

Verena Grundler and Karl Gademann

Zusammenfassung: Steigende Temperaturen, extensive Landwirtschaft sowie die Globalisierung begünstigen den unkontrollierbaren Wuchs von Cyanobakterien (alte Bezeichnung: Blaualgen). Da sie zu den ältesten Lebensformen auf unserem Planeten zählen, besiedeln sie viele Habitate, wobei die Mehrheit in Gewässern zu finden ist. Unglücklicherweise produzieren diese Organismen eine Reihe von verschiedenen Toxinen, weswegen von einer Algenblüte unter Umständen große Gefahren für Mensch und Tier ausgehen können. Ein kurzer Überblick über diese interessante, jedoch teilweise gefährliche Spezies wird im Rahmen dieser Arbeit gegeben.

Abstract: Rising temperatures, extensive agriculture together with globalization favor the uncontrolled growth of cyanobacteria, formerly called blue-green algae. These organisms belong among the oldest life forms on our planet populate many habitats, in particular marine and freshwater environments. Unfortunately, those organisms are known to produce various toxic compounds, wherefore a bloom can cause great danger for humankind and animals. This article gives a short overview over this interesting, yet dangerous species.

Key words: Toxic compounds, cyanobacteria, phytoplankton bloom, Microcystin-LR, Anatoxin-a, Saxitoxin, Nodularin.

Introduction

Over the last years, warning signs reporting danger of intoxication in shore areas have been emerging in many areas over the world (Figure 1). In many cases, people witnessed symptoms of nausea and dizziness while being exposed to a refreshing breeze from the ocean. More severe cases, from skin itching to acute intoxication, have been reported after immersion and ingestion of contaminated water.

These symptoms are often caused by certain types of microorganisms, such as cyanobacteria (formerly called blue-green algae.) These prokaryotic phototrophs are among the oldest species on our planet, as indicated by fossil records from western Australia, and they have been postulated to exist on earth since 3.5 billion years ago (Schopf and Packer, 1987). Cyanobacteria can be found in many habitats on earth, in particular freshwater, brackish as well as saline water environments (Carmichael and Li, 2006). In addition, they can adapt and survive environmental stress, such as dramatic temperature changes, dry periods and very high and low temperatures. For example, cyanobacteria are able to grow in polar regions, in antarctic terrestrial environment or in hot springs in Yellowstone National park at 74 °C (Briand et al., 2004; Jungblut et al., 2009; Reichwaldt and Ghadouani, 2012; Tang et al., 1997; Ward et al., 1998). Even extreme hostile environments such as volcanic ash and desert sand can be cultivated by cyanobacteria (Dor and Danin, 1996; Gerasimenko et al., 2013). Some species as *Synechococcus/Synechocystis* spp. can live in symbiosis with dinoflagellates, whereas others associate with fungi and plants (Gordon et al., 1994; Hyvarinen et al., 2002; Meeks, 1998). This synergy is often due to the remarkable ability of cyanobacteria to fix nitrogen from the atmosphere, which then is further enzymatically converted into ammonium derivatives (Herrero et al., 2001; Latysheva et al., 2012). Responsible for this process are specialised cells, the so-called heterocysts, which have a large and round shape and possess thick cell walls with oxygen-binding glycolipids (Pitois et al., 2000). As energy metabolism for life, these photoautotrophic organisms use photosynthesis and likewise, this circumstance has been suggested to play a key role in evolution of life on Earth as cyanobacteria are assumed to have generated the oxygen in the atmosphere (Dismukes et al., 2001; Stanier and Bazine, 1977).

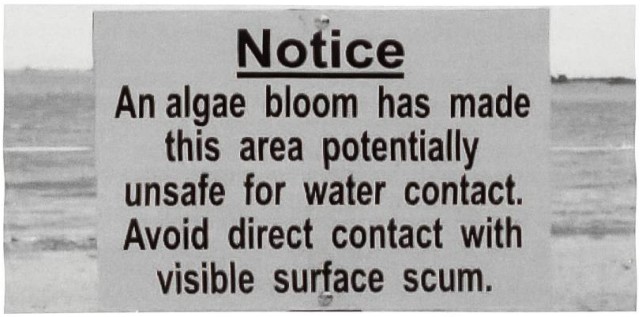

Fig. 1: Marion Reservoir, Kansas.
Photo Credit: Jennifer L. Graham USGS

Cyanobacteria are unicellular to multicellular prokaryotes, which belong to the *Bacteria* domain, and these multicellular bacteria can be further categorized in filamentous, undifferentiated and differentiated species (Flores et al., 2006; Rokas, 2008). The multicellular bacteria are able to communicate intercellularly, to differentiate and form cell-cell adhesion (Flores and Herrero, 2010). An example for an unicellular bacteria would be *Microcystis aeruginosa* (Figure 2A). An example of a multicellular filamentous and undifferentiated bacteria is represented by *Planktothrix* and *Anabaena sp.* belongs in the group of multicellular differentiated cyanobacteria (Schirrmeister et al., 2011).

As any species, cyanobacteria produce a great variety of secondary metabolites such as carotenoids, fatty acids, lipopeptides, polysaccharides and as well as different bioactive molecules. A large number of cyanobacterial compounds are peptides or contain a peptic substructure and, so far, more than 600 members have been described (Dixit and Suseela, 2013; Welker and Döhren, 2006). A large structural diversity exists within these compounds, and the incorporation of unusual or modified amino acids is often found. As cyanobacteria have populated earth for a long time period, it is not surprising that many of the above mentioned molecules show significant bioactivity. It has been reported that some secondary metabolites of cyanobacteria show anti-

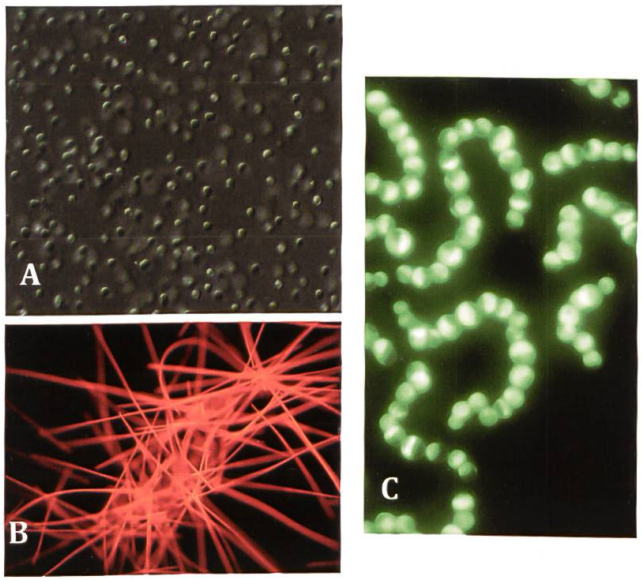

Fig. 2:
A) Microcystis aeruginosa
B) Planktothrix rubescens;
Photo Credit: Thomas Posch
C) Nostoc sp.;
Photo Credit: Bettina Eugster & Esther Kohler.

cancer, cytotoxic, antibiotic, antifungal and antiviral activity, as well have multi-drug resistance, antimycotic, immunosuppressive and anti-malarial features (Burja et al., 2001). As the compounds produced by cyanobacteria can by characterized by high chemical stability and water solubility, they can be considered also as interesting candidates for medical applications, such as the anticancer agent dolastatin, which failed however in clinical trials due to toxicity effects (Luesch et al., 2001; Simmons et al., 2005).

In addition to these compounds that may have a positive impact by being potentially used as drugs, cyanobacteria produce several highly toxic molecules, the cyanotoxins, which pose a danger to humans, animals and the ecosystem alike. These toxins are also characterized by a high chemical stability and water solubility and thus have the potential for severe intoxication of humans and animals (Carmichael, 1992). In fact, due to eutrophication, cyanobacteria often prosper in aquatic environment and cause dangerous blooms all over the world. In addition, climate change and therefore rising temperatures on our planet, the growth of toxic cyanobacteria might be further facilitated and become an even greater problem (Paerl and Huisman, 2008; Pitois et al., 2000). Toxic blooms mainly affect warm regions as Africa or South America, where every year many people are effected and water supply is endangered (Oberholster et al., 2005; Lagos et al., 1999). In addition, Europe is also struggling with the problem of toxic cyanobacteria, for example in the Baltic Sea (Figure 3), where the occurrence of blooms increased over the past years (Kahru et al., 2007). These phenomena are visible from space, as an image from a satellite clearly demonstrates (Figure 3). Furthermore, European lakes are as well affected by increasing density of toxic cyanobacteria (Eiler et al., 2013; Kurmayer and Gumpenberger, 2006).

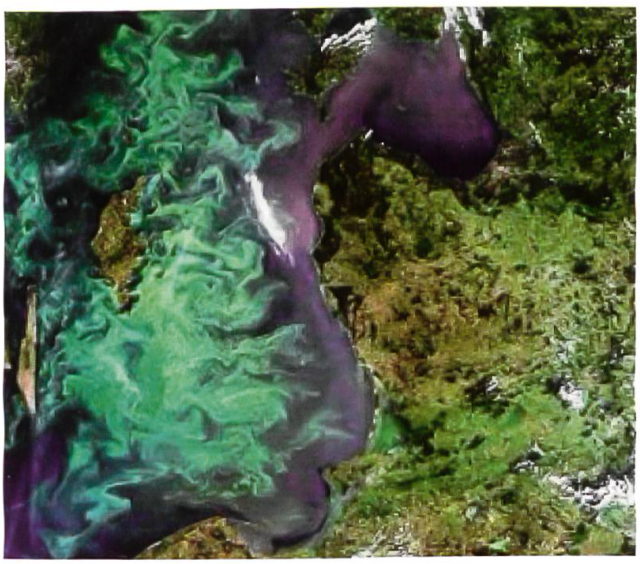

Fig. 3: Phytoplankton bloom in the Baltic Sea.
Photo Credit: European Space Agency, ESA

Various toxins of cyanobacteria

A major problem occurs after the collapse of those blooms. This way various toxins get released and thereby pollute fresh and marine water, which creates a serious threat to water supply, public health, livestock and wildlife (Paerl et al., 2001). Another danger for health is inflicted by direct contact with poisoned water through swimming in a lake with a cyanobacteria bloom or drinking contaminated water. Besides this, another serious problem poses the circumstance that these toxins can enter the food chain via accumulation in fish, mussels or shell fish and on this way find their way into humans (van Apeldoorn et al., 2007).

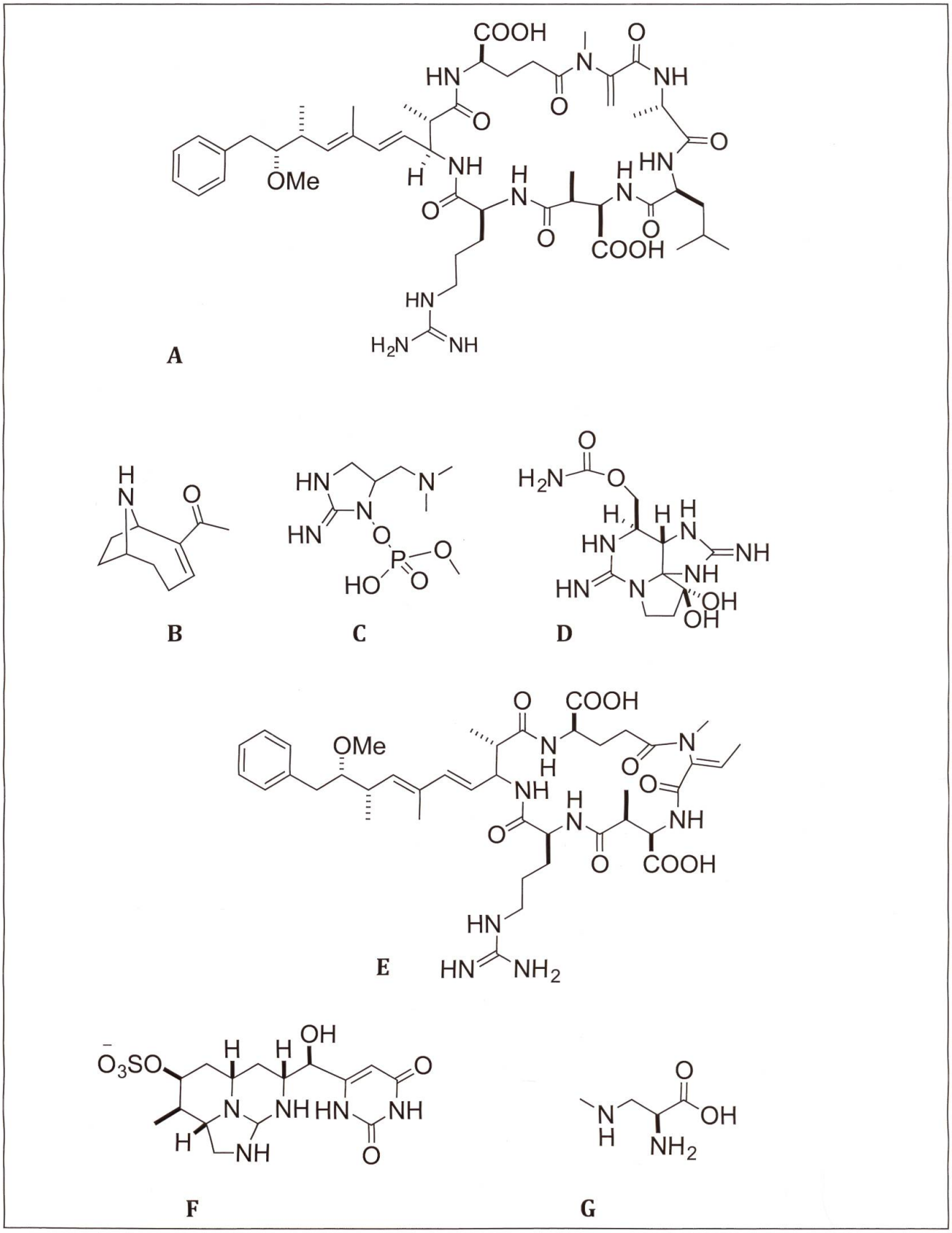

Fig. 4: A) Microcystin-LR; **B)** Anatoxin-a; **C)** Anatoxin-a(s); **D)** Saxitoxin; **E)** Nadularin; **F)** Cylindrospermo–psin; **G)** ß-*N*-methylamino-L-alanine

Over the past years, many cases of poisoning by cyanobacteria were reported, whereby the regular intoxication occurs via oral intake, although other uptake mechanisms are also possible, such as inhalation or skin contact (Stewart et al., 2006). The main cases of intoxication happen with animals, for example at a lake in Canada where approximately 1000 bats died after ingesting cyanobacterial polluted water (Pybus et al., 1986). In addition, also humans suffer from cyanobacterial poisoning. A tragic accident of acute intoxication with microcystin containing water occurred in Brazil in the year 1996, where dialysis patients were treated with contaminated water and this conjuncture lead to the death of 52 people (Azevedo et al., 2002). A more recent case of intoxication of a young person was reported from Argentina, who dived in a lake with a cyanobacteria bloom and thus showed the clear symptoms of intoxication, like e.g. nausea and respiratory distress (Giannuzzi et al., 2011). The main toxin, which has been suggested to be responsible for these poisonings is Microcystin-LR, which belongs to a wide group of cyclic peptides, the microcystins with a great variety of 80 derivatives known to date (Zeller et al., 2011). Besides the general core structure *cyclo*(-D-Ala-Xaa-D-MeAsp-Yaa-Adda-D-Glu-Mdha-), two diverse amino acids (Xaa, Yaa) lead to this great structural diversity (Mikalsen et al., 2003).Within this compound class, MC-LR (Xaa = Leu, Yaa = Arg) has an exceptional position as most studied microcystin, due to its high LD_{50} value of 50 μgkg^{-1} (Rodríguez et al., 2008). In fact it is one of most potent and toxic compound produced by cyanobacteria and on this account the WHO set a guideline value of MC-LR in drinking water of 1 μgL^{-1} (Gupta et al., 2003; WHO, 2003).

In addition to the hepatoxic effect in humans through inhibition of protein phosphatases 1 and 2A, chronic consumption of MC polluted water can cause liver cancer (MacKintosh et al., 1990; Ueno et al., 1996; Fujiki and Suganuma, 2011). Moreover, MC-LR seems to cause DNA damage, apoptosis, cell-signaling disruption and endoplasmatic reticulum stress (Campos and Vasconcelos, 2010; Christen et al., 2013; Žegura et al., 2008). The known transport of MCs in the cell is the organic anion polypeptide transporter (OATP), which is present e.g. in human hepatocytes (Fischer et al., 2010; Magalhães et al., 2003). In aquatic organisms, which are especially exposed to cyanobacterial toxins, microcystins shows also toxic effects. For example, in *Daphnia magna,* which is an important grazer using cyanobacteria as a food source, reproduction is disturbed, which is reflected in higher mortality and suppressed development of the offspring (Dao et al., 2010; Lürling, 2003; Ortiz-Rodríguez et al., 2012). In fish and other invertebrates, the toxin leads to hepatotoxic effects and accumulation in tissues (Cazenave et al., 2005). Nevertheless, although intensive research on MC-LR has been carried out since decades, the reason for the strong toxicity is not yet fully understood.

In addition to microcystins, cyanobacteria produce a wide range of other potent, various toxins and the structural variety of these toxic compounds reaches from peptides, linear or heterocyclic, to lipid compounds (Bláha et al., 2009). Based on their toxicity effect and their mode of action and uptake, they can be categorized into different groups; the neurotoxins, the cytotoxins, the dermatotoxins, the irritant toxins and the hepatoxins (Wiegand and Pflugmacher, 2005).

For example, the neurotoxic compounds anatoxin-a, anatoxin-a(s) and saxitoxin are among the most toxic compounds produced by cyanobacteria, but display, due to their rare occurrence and therefore reduced exposure, a minor danger to humans when compared to the hepatoxins such as the microcystins and nodularins (Mankiewicz et al., 2003). Anatoxin-a is a bicyclic, secondary amine, which is a strong agonist at the nicotinic acetylcholine receptors (Devlin et al., 1977). Toxicity symptoms reveal quickly after consumption of contaminated water and are manifested in paralysis, tremors, convulsions and death (Rogers et al., 2005). Anatoxin-a(s) constitutes an organophosphate, structurally not related to anatoxin-a, which irreversibly blocks acetylcholinesterase and enhances salivation (Dittmann et al., 2013; Matsunaga et al., 1989). Furthermore, the administration of pure toxin to mice leads to fast death and respiratory failure

within 240 min. (Onodera et al., 1997). Saxitoxin, produced by freshwater cyanobacteria as well as marine dinoflagellates, is a strong neurotoxin, which accumulates in the food chain and causes paralytic shellfish poisoning (Pearson et al., 2010). Symptoms occur rapidly after intake of poisoned food and are manifested as preliminary numbness around the lips, which later affects the whole body and can lead to respiratory paralysis (Cusick and Sayler, 2013). Its chemical core structure is a trialkyl tetrahydropurine, whereas 30 derivatives are known (Llewellyn, 2006). Another example for a cyanobacterial compound is cylindrospermopsin, a cytotoxic and genotoxic alkaloid, which covalently binds to DNA and shows a toxicity effect in liver, kidney and lung (Moreira et al., 2013). Additionally, it is demonstrated to be carcinogenetic and has evidence to inhibit protein synthesis (Falconer and Humpage, 2006). The strong liver toxicity effect is explained by a oxidation of the toxin by CYP450, which leads to a more potent toxic product (Runnegar et al., 1995). Additional toxins are, for example, lyngbyatoxin-a, debromoaplysiatoxin and aplysiatoxin, representing common dermatotoxins (Mankiewicz et al., 2003). The typical common clinical symptom of these toxins are dermatitis, complemented by tumor promotor activity and protein kinase C activation in the case of debromoaplysiatoxin and aplysiatoxin, as well as gastrointestinal inflammation for lyngbyatoxin-a (Cardellina et al., 1979; Mynderse et al., 1977). Another class of toxins are the irritant toxins. The lipopolysaccharides fall in this category, as they represent a crucial component of the cell wall of all bacteria (Bláha et al., 2009). The toxicity effect occurs after contact with these toxins and appears as allergenic response, gastroenteritis and inflammation (Mur et al., 1999). Due to the less toxic effect in comparison to other bacteria, the importance of these toxins is slightly smaller classified then the abovementioned (Keleti and Sykora, 1982). The last compound class are the hepatoxins, which represent the most studied toxins so far (see the discussion on MC above). As mentioned above, cyanobacteria are known to be hepatotoxic and exposure to blooms cause typical clinical symptoms for liver damage, such as increased liver weight, haemorrhage and blood pooling and this deep red coloration, as well as the enhanced activity of glutamate pyruvate transaminase/alanine aminotransferase, lactate dehydrogenase and alkaline phosphatase (Kumar et al., 2006). The two common hepatoxins are represented by the microcystins (see above) and nodularin, whereas this toxin shows a similar toxicity effect as microcystin (Zurawell et al., 2005). The characteristic feature of both compounds is a strong inhibition effect of protein phosphatase 1 and 2A, as well as promotion of liver cancer (MacKintosh et al., 1990; Ullah, 2011). Nodularin has a similar structure as microcystin, containing the Adda function, D-Glu, D-MeAsp, L-Arg and MeDhb and thus leading to similar toxic effects. In contrast to the microcystins, however, a much smaller number has been described so far, as only seven isoforms of this toxin have been reported (Pearson et al., 2010).

In addition to the above-mentioned categorized toxins, cyanobacteria produce other classes of natural products, which share structural similarity and biological effects. These compounds can be further classified regarding their general core structure into aeruginosins, microginins, anabaenopeptins, cyanopeptolins, microcystins, microviridins and cyclamides (Welker and Döhren, 2006). A large group of these toxins are the aeruginosins, which are trypsin-type serine protease inhibitors produced by *microcystis* and *oscillatoria* (Murakami et al., 1995; Shin et al., 1997). More than 40 variants are known up to date, and the characteristic core structure of this peptide class is determined by the presence of the unusual amino acid 2-carboxy-6-hydroxyoctahydroindole (Choi) and the hydroxyphenyllactic acid (Hpla), further a variable amino acid (Leu, Ile, Phe, or Tyr) and an arginine derivative (Elkobi-Peer et al., 2013). Another group of serine inhibitors are the cyclic peptides belonging to the group of cyanopeptolins, which are characterized by the amino acid 3-amino-6-hydroxy-2-piperidone (Ahp) (Martin et al., 1993). These toxins show a great structural diversity based on the variation of several various amino acids as well as derivatization or chlorination (Matern et al., 2001; Weckesser et al., 1996). The synthesis of these large biomolecules is mostly performed

by large enzyme complexes, the nonribosomal peptide synthetases (NRPS) or a combination of polyketide synthases and NRPSs systems (Schwarzer et al., 2003). The gene clusters of those synthetases contain highly conserved NRPS operons, containing the mcyABC gene cluster, within the different strains and thus suggest a horizontal gene transfer as an explanation for the similarity of the produced compounds (Mikalsen et al., 2003; Rounge et al., 2008). Recently, B-N-methylamino-L-alanine, a neurotoxic amino acid produced by cyanobacteria, received a lot of attention as it is considered to be a trigger for human neurological diseases such as Amyotrophic Lateral Sclerosis, Parkinson and Alzheimer's disease (Banack et al., 2010; Pablo et al., 2009). The disease seems to be initiated by an overactivation of neuroexcitatory glutamate receptors, which leads to damage of the neurons (Vyas and Weiss, 2009).

Ecological and biological role of cyanobacterial toxins

Despite all the research done on cyanobacterial toxins, the ecological and biological role of cyanobacterial toxins is still under investigation, whereby the recent production purpose is mainly linked with the protection against grazers (Blom et al., 2001; Lürling, 2003; Wilson et al., 2006). In contrast to this theory, phylogenetic analyses suggests that the synthetase genes developed simultaneously with the housekeeping genes and thus are older than grazers (Rantala et al., 2004). Another purpose is assumed to act as allelochemicals to repel other photosynthetic competitors or the possibility of a physiological function as for example cell signalling or iron scavenging is also thinkable, since the long existence of these compounds and the effort of production suggest a more important role in the organism (Berry et al., 2008; Holland and Kinnear, 2013). In any case the existence of mutants of cyanobacteria, which lost the ability to produce microcystin by inactivation of the microcystin synthetase through mutations in the gene cluster, could be an hint to exclude the toxic purpose of microcystin (Christiansen et al., 2008). But this issue is getting even more complex facing the fact that non-toxic cyanobacteria exist and also form blooms without the missing disadvantage in growth compared to their poisonous siblings (Merel et al., 2013; Ostermaier and Kurmayer, 2009). Therefore, the missing «final explanation» for the purpose of the toxin production renders toxic cyanobacteria still a mysterious species.

Conclusions

In conclusion, the occurrence of toxic cyanobacteria in marine and freshwater environments will likely increase in the near future, due to climate change and eutrophication. As shown in this highlight article, the presence of a manifold of toxic compounds in these phototrophic organisms continues to pose a challenge to a variety of recreational, nutritional and industrial activities. As the mechanism of action for toxicity for many compounds is yet poorly understood, continuous research activities are needed to deepen our understanding of the chemical potential of cyanobacteria.

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Verena Grundler and Karl Gademann
Department of Chemistry
University of Basel
St. Johanns-Ring 19
4056 Basel