REVIEW / SYNTHÈSE

Cyanobacterial bioactive molecules — an overview of their toxic properties

Pranita Jaiswal, Pawan Kumar Singh, and Radha Prasanna

Abstract: Allelopathic interactions involving cyanobacteria are being increasingly explored for the pharmaceutical and environmental significance of the bioactive molecules. Among the toxic compounds produced by cyanobacteria, the biosynthetic pathways, regulatory mechanisms, and genes involved are well understood, in relation to biotoxins, whereas the cytotoxins are less investigated. A range of laboratory methods have been developed to detect and identify biotoxins in water as well as the causal organisms; these methods vary greatly in their degree of sophistication and the information they provide. Direct molecular probes are also available to detect and (or) differentiate toxic and nontoxic species from environmental samples. This review collates the information available on the diverse types of toxic bioactive molecules produced by cyanobacteria and provides pointers for effective exploitation of these biologically and industrially significant prokaryotes.

Key words: cyanobacteria, bioactive molecules, cyanotoxins, NRP (non-ribosomal peptide), biocontrol agent.

Résumé : Les effets allélopathiques des cyoanobactéries sont de plus en explorés pour identifier les molécules bioactives importantes d'un point de vue pharmaceutique ou environnemental. Parmi les composés toxiques produits par les cyanobactéries, les biotoxines sont bien connues quant aux voies, aux mécanismes régulateurs et aux gènes impliqués dans leur biosynthèse, alors que les cytotoxines sont moins étudiées. Une variété de méthodes de laboratoire ont été développées afin de détecter et d'identifier les biotoxines de l'eau et les agents qui en sont responsables; elles diffèrent grandement quant à leur degré de sophistication et à l'information qu'elles génèrent. Des sondes moléculaires directes sont aussi disponibles afin de détecter et différencier les espèces toxiques des espèces non toxiques d'échantillons environnementaux. Cette revue collige l'information disponible sur les différents types de molécules bioactives toxiques produites par les cyanobactéries et fournit des pistes pour exploiter de façon efficace ces procaryotes significatifs d'un point de vue biologique et industriel.

Mots-clés : cyanobactéries, molécules bioactives, cyanotoxines, peptide non ribosomal, agent de contrôle biologique.

[Traduit par la Rédaction]

Introduction

Cyanobacteria are an ancient group of simple microorganisms, with characteristics in common with both bacteria and algae. They resemble bacteria in their prokaryotic cellular organization but exhibit oxygen-evolving photosynthesis similar to algae and higher plants. They are employed as model systems for understanding various physiological processes, besides serving as important links in the evolutionary and (or) phylogenetic classification of organisms. They were the first organisms to carry out oxygenic photosynthesis and played a major part in the oxygenation of the atmosphere of earth. Their remarkable ecological diversity combined with very simple metabolic requirements is responsible for their success as a group in a wide range of aquatic habitats. Also, their unique physiological characteristics and high adaptive ability under a wide range of environmental conditions leads to their proliferation as excessive masses in aquatic habitats, often dominating other aquatic flora and fauna. The production of water blooms is a widespread phenomenon, which has been reported from different parts of world, and poses a considerable threat not only to the flora and fauna but also significantly to the health and welfare of human beings.

Biochemical processes in living cells are of 3 main types: basal (providing energy and raw materials for cell functions), synthetic (involved in replication and vegetative growth), and secondary (involved in the utilization of substrates to give a variety of products often specific for a given organism). Among these processes, the maximum chemical diversity is observed in the secondary metabolites,

Received 1 December 2007. Revision received 2 April 2008. Accepted 11 April 2008. Published on the NRC Research Press Web site at cjm.nrc.ca on 8 August 2008.

P. Jaiswal and R. Prasanna. Centre for Conservation and Utilization of Blue Green Algae (CCUBGA), Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi 110012, India.
P.K. Singh.[1] Department of Botany, Banaras Hindu University, Varanasi, India.

[1]Corresponding author (e-mail: pksalgae@yahoo.co.in).

 doi:10.1139/W08-034

such as cyanotoxins, which not only provide heterogeneity but also serve as inter-/intra-species level markers for organisms. A heavy water bloom formed under adequate light and temperature can also serve as a rich source of secondary metabolites of novel chemical and molecular structures (Zingone and Enevoldsen 2000; Welker and Von Dohren 2006; Volk 2007). Many of these biomolecules have pharmaceutical importance and include hepatotoxic (liver damaging), neurotoxic (nerve damaging), cytotoxic (cell damaging) compounds, and toxins responsible for allergic reactions (Carmichael 1994; Volk and Mundt 2006). In recent years, there has been a tremendous enhancement in our knowledge regarding their biological significance, especially those produced by cyanobacteria, as they have proved to be exciting molecules with immuno-modulatory, bioregulatory, and therapeutic potential (Namikoshi and Rinehart 1996; Burja et al. 2001; Singh et al. 2005*b*).

The first report on cyanobacterial toxins dates back to May 1878 when George Francis of Australia reported that the blue-green alga *Nodularia spumigena* formed a thick scum-like green oil paint on the Murray River, and its growth rendered water "unwholesome" for cattle and other animals drinking at the surface, bringing on a rapid and sometimes terrible death. Since then, there have been several reports on cyanobacterial toxins and their ecological and economic impacts, besides the sociocultural implications (Sivonen 1990; Carmichael 1994; Ray and Bagchi 2001; Ghasemi et al. 2003; Agrawal et al. 2005; Wiegand and Pflugmacher 2005; Volk 2006). Until the late 1990s, there have been no definite reports on human death due to cyanotoxins. Pouria et al. (1998), however, reported that 126 patients suffered from toxic hepatitis due to the use of contaminated water for haemodialysis, among which 60 died. Immunoassays confirmed lethal doses of cyanotoxins in the liver of the patients. The toxicity level of any water body contaminated with cyanobacteria depends on various factors, e.g., cellular concentration, type of toxins, biomass concentration, mode of exposure, and susceptibility of victim, notably age, sex, weight, and species (Carmichael 1994).

Despite the availability of information on cyanotoxins, a comprehensive evaluation of various facets of their production, i.e., environmental factors, detection techniques, and genetic basis, is not currently available to our knowledge. The major focus of our review is therefore to compile and analyse the available information on cyanotoxins and discuss their ecological significance.

Factors affecting toxicity

Toxin production by cyanobacteria appears highly variable both within and between blooms (Codd and Bell 1985) and toxicity not only varies between strains but among clones of same isolates (Carmichael 1994; Utkilen and Gjolme 1992). In addition, few strains produce 3 or more toxins with the relative proportions being influenced by environmental factors (Wicks and Thiel 1990; Carmichael 1994; Utkilen and Gjolme 1992).

Growth phase

Culture age significantly affects toxin production in cyanobacteria. Gentile reported for the first time in 1971 about leakage of toxin at mid-exponential phase of growth of *Microcystis aeruginosa* (Gentile 1971). Since then, there have been many reports regarding optimum toxin production and release by *M. aeruginosa* at late exponential phase of growth (Codd and Poon 1988; Carmichael 1994). Similar results were obtained by Ray and Bagchi (2001) on *Oscillatoria* sp. They observed that algicide started appearing in the medium at mid-exponential phase, which showed a positive relation with biomass yield. The differential concentration of algicide in the medium and inside the cells led them to conclude that the compound is secreted by an efflux mechanism rather than leaking out of cells. Dias et al. (2002) reported that in *Aphanizomenon* sp. strain LMECYA 31, the amount of extracellular toxin increased with culture time, indicating that toxins are released in water through cell lysis and may be expected to remain in water upon collapse of the toxic bloom or removable by water treatment. Patterson and Bolis (1993) reported a rapid decrease in the scytophycin content of *Scytonema ocellatum* in newly inoculated cultures, which suggests that scytophycin is continuously metabolized. Rapala et al. (1997) reported that in *Anabaena*, culture age is the most important factor causing the release of toxins. Microcystin and anatoxin-a are largely retained within the cell when the conditions are favorable for growth. The amount of microcystin in the culture increases during the logarithmic growth phase and is highest in the late logarithmic phase. Maximum anatoxin-a concentration was found during the logarithmic phase of growth (Sivonen 1996). Volk (2007) reported variation in exometabolites excreted by *Nostoc insulare* with culture age. During linear growth a nontoxic compound was excreted in the medium, whereas during stationary phase, antimicrobial and cytotoxic exometabolites were also present in the extracellular medium.

Nutritional factors

Toxin production in cyanobacteria is affected by various nutritional factors like nitrogen (N) and phosphorus (P) concentration. Codd and Poon (1988) found 10 times less toxin in *M. aeruginosa* cultures as compared with reference cells when the nitrogen source was removed. Watanabe and Oishi (1985) also observed a slight reduction in toxin production with lower nitrogen levels, and Sivonen (1990) showed a direct relationship between toxin production and nitrate concentration in *Oscillatoria agardhii.* Since *M. aeruginosa* and *O. agardhii* are non-N_2 fixers, the stimulation in toxin production in the presence of enhanced levels of inorganic combined nitrogen sources can be directly correlated with the peptide nature of their toxins.

Toxin production is favoured by a low level of phosphorus present in the medium (Watanabe and Oishi 1985; Sivonen 1990). Sivonen (1990) reported that lower levels of phosphorus are needed for toxin production and a saturation level of 0.4 mg P/L was recorded in this study. Utkilen and Gjolme (1992) reported that phosphate-limiting conditions had no effect on toxin production by *M. aeruginosa*. Rapala et al. (1993) reported no significant variation in the production of anatoxin-a due to the concentration of P in the medium. On the other hand, Oh et al. (2000) observed that more P in the culture medium stimulates growth and toxin production by *M. aeruginosa*. The role of environmental

factors, such as pH, light and temperature, and P levels, on the growth and production of biocidal compounds by *Anabaena* sp. and *Calothrix* sp. was also investigated (Radhakrishnan 2006). The diameter of the inhibition zone was largest when extracellular filtrates of cultures of *Anabaena* sp. and *Calothrix* sp. grown at a 2-fold higher concentration of P (1.4 mg/L compared with 0.7 mg/L in BG 11 medium) were employed in disc diffusion assays using cyanobacteria and phytopathogenic fungi as test organisms. Repka et al. (2004) also reported maximum toxin production by a cyanobacterium in 13-day-old culture of *Anabaena* sp. strain 90 in the presence of 2.6 mg/L phosphate concentration. However, Ray and Bagchi (2001) reported that algicide production by *Oscillatoria latevirens* was negatively regulated with phosphate. They also analysed the effect of sulphur, magnesium, calcium, and hydrogen on growth and secondary metabolite production by cyanobacteria and found that although S did not show any effect on growth and algicide production, a decline in magnesium concentration (within the range that permitted growth) enhances the algicide production and its inhibitory activity. However, calcium was required by the strain for growth, although it did not have any effect on algicidal activity. Dias et al. (2002) reported that changing phosphate levels results in a change in the type of toxin produced by cyanobacterium *Aphanizomenon* sp. Evidently, P level has a significant influence on the toxin production by cyanobacteria, but a differential response is observed among different cyanobacterial strains and genera. This may be attributed to the quantitative and qualitative differences in the specific requirements of the strains, and no general relationship can be attributed in terms of the influence of nutrients, especially phosphorus.

Environmental factors

Most of the preliminary work regarding the influence of environmental factors on toxin production by cyanobacteria was done using *M. aeruginosa* (Watanabe and Oishi 1985; Van der Westhuizen et al. 1986; Sivonen 1990). Watanabe and Oishi (1985) found that light intensity is the primary factor for toxin production, and low light intensity suppressed its production. They observed a 4-fold increase in *M. aeruginosa* toxicity when light intensity was increased from 7.53 to 30.1 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Van der Westhuizen and Eloff 1985). Van der Westhuizen et al. (1986) observed lower toxin production at very low and high light intensities with *M. aeruginosa*. However, Wicks and Thiel (1990) reported only small differences in toxicity at light intensities of 37 and 270 $\mu E \cdot m^{-2} \cdot s^{-1}$. A positive correlation was found between solar radiation and toxicity of *M. aeruginosa* under natural conditions. On the contrary, Codd and Poon (1988) reported that light intensities of 5–50 $\mu E \cdot m^{-2} \cdot s^{-1}$ had no significant influence on toxin production in a strain of *M. aeruginosa*. Utkilen and Gjolme (1992) reported an increase in toxin production rate up to 40 $\mu E \cdot m^{-2} \cdot s^{-1}$, and any further increase in light intensity resulted in a decrease in toxicity. They found that the toxin produced by this strain is a small peptide, and the ratio of toxin to protein increases up to 40 $\mu E \cdot m^{-2} \cdot s^{-1}$. But in contrast to toxicity, this ratio is almost unaffected by any further increase in light intensity. Hence, they concluded that toxin synthesis increased faster than general protein synthesis at light intensities between 20 and 40 $\mu E \cdot m^{-2} \cdot s^{-1}$. They showed that both toxicity and the ratio of toxin to protein were slightly enhanced by both red and green light as compared with white light. However, they concluded, a change in light quality had a minor effect on toxicity but may directly affect growth. The decrease in toxin production at high light intensities may also be caused by an accumulation of polysaccharides (Utkilen and Gjolme 1992). Radhakrishnan (2006) observed that high light intensity (5000 lx or 90–100 μmol photons$\cdot m^{-2} \cdot s^{-1}$ and temperature (40 ± 2 °C) enhanced the algicidal and fungicidal activity of the extracellular filtrates of the *Anabaena* sp. and *Calothrix* sp. Such contradictory reports regarding the effect of light intensities were explained by Sivonen (1990) who attributed them to differences in light sources, differences in culture media used, and differences in toxin detection method used. It can also be concluded that the differential behavior of a strain and its optimal light requirements for growth may have a direct correlation with toxicity.

The optimum temperature for the production of cyanobacterial toxins also shows conflicting results. Gorham (1964) found that the toxicity of *M. aeruginosa* to mice was greater after growth at 25 °C than at 20 °C, although it decreased at 30 °C to an intermediate level. Van der Westhuizen and Eloff (1983, 1985) and Watanabe and Oishi (1985) reported a decrease in the toxicity of *Microcystis* isolates with increasing growth temperature. Ressom et al. (1994) found that most strains of *M. aeruginosa* produce less toxin at upper and lower limits of temperature optima for the species. The formation of cyclic peptides by *Microcystis* was significantly reduced at a temperature range of 30–35 °C (Gorham 1964; Watanabe and Oishi 1985). However, the optimum temperature for the formation of anatoxin (Peary and Gorham 1966), saxitoxin (Gentile and Maloney 1969), and cyclic peptide hepatotoxin by *O. agardhii* (Sivonen 1990) was the same as the optimum temperature for growth of the strain. Sivonen (1990) reported that toxin production by a strain of *O. agardhii* was maximum at 25 °C, whereas another strain produced a similar amount of toxin at temperatures between 15 and 25 °C. The lowest amount of toxin produced by both *O. agardhii* strains was at 30 °C. Similar results indicating a lack of significant influence of light intensity or temperature and nitrate concentration on algicide production were obtained by Jaiswal et al. (2007). In general, a temperature range of 20–25 °C is best suited for toxin production in most cyanobacterial strains (Codd and Poon 1988; Rapala et al. 1997; Rapala and Sivonen 1998). Toxin production at different temperatures seems to be strain dependent.

Toxin production by cyanobacteria is also influenced by pH of the medium (Van der Westhuizen and Eloff 1983; Ray and Bagchi 2001). Van der Westhuizen and Eloff (1983) reported that although the cyanobacterium *M. aeruginosa* exhibited a maximum growth rate at pH 9, the cells were more toxic at higher and lower pH values, where they grew more slowly. Ray and Bagchi (2001) observed a negative regulation of pH and toxin production in *O. latevirens*. A pH of 8 was found to be most optimal (Table 1) for *Calothrix* sp. in terms of growth and biocidal activity against 2 cyanobacterial and 2 fungal strains (Radhakrishnan 2006). However, Jaiswal et al. (2007) showed that there is no sig-

Table 1. Biocidal activity of cell-free filtrates of *Calothrix* sp. grown for 2 weeks at different pH regimes.

	Biocidal activity measured as zone of inhibition (mm) on the lawn of:			
pH	*Synechococcus* sp.	*Synechocystis* sp.	*Rhizoctonia bataticola*	*Pythium aphanidermatum*
8.0±0.2	33.66 (1.5391)[a]	32.66 (1.527)[a]	13.0 (3.6725)[b]	7.33 (3.3408)[b]
9.0±0.2	14.66 (1.1895)[a]	7.0 (0.7068)[a]	0 (0.7071)[b]	0 (0.7071)[b]
10.0±0.2	0 (0)[a]	0 (0)[a]	0 (0.7071)[b]	0 (0.7071)[b]
Critical difference (P = 0.05)	0.1028	0.7067	0.1571	0.1004

[a]Figure in parentheses is the log transformed value (X + 1.0).
[b]Figure in parentheses is the square-root transformed value (X + 0.5).

nificant effect of pH on secondary metabolite production by the cyanobacterium *M. aeruginosa.*

In general, the effect of environmental and nutritional factors seems to be strain specific and growth linked. However, the kinetics of intracellular and extracellular release needs to be directly analysed to understand the cellular targets of the influencing factors.

Types of cyanotoxins

Cyanobacterial toxins can be divided into 2 main categories, based on the type of bioassay used to screen their activity. (*i*) Biotoxins cause acute lethal poisoning and are assayed using small animals, e.g., mice and aquatic invertebrates. (*ii*) Cytotoxins are not highly lethal to animals but show more selective bioactivity and are assayed using cultured mammalian cell lines, especially tumor cell lines.

Biotoxins

The most studied algal biotoxins are divided in 3 classes based on chemical structure: cyclic peptides, alkaloids, and lipopolysaccharides (LPS). The commonly known biotoxins and their mode of action, structure, and LD_{50} are presented in Table 2.

Cyclic hepatotoxic peptides

Cyclic hepatotoxic peptides are the most common offenders worldwide in cases of waterborne diseases caused by toxic cyanobacteria. The major cyanotoxins causing death and illness in animals are the peptide hepatotoxins or liver-affecting toxins (Carmichael 1994). Nodularins and microcystins are cyclic peptides containing 5 and 7 amino acids, respectively, with the 2 terminal amino acids of the linear peptide being condensed (joined) to form a cyclic compound. The cyclic peptides are comparatively large natural products, with a molecular weight of 600–1100, but are small compared with many other cellular oligopeptides and polypeptides (molecular weight > 10 000).

Microcystins

Microcystins are a group of cyclic heptapeptide (7 amino acids) hepatotoxins produced by a number of cyanobacterial genera, the most notable of which is the widespread *Microcystis* from which the toxins take their name. Approximately 70 different structural analogues of microcystins have been identified to date (Sivonen and Jones 1999; Babica et al. 2006). Microcystins consist of a 7-membered peptide ring, which is made up of 5 non-protein amino acids and 2 protein amino acids. It is these 2 protein amino acids that distinguish microcystins from one another, while the other amino acids are more or less constant between variant microcystins. Using amino acid single letter code nomenclature, each microcystin is designated a name, depending on the variable amino acids that complete their structure (Fig. 1). The most common and potently toxic microcystin-LR contains the amino acids leucine (L) and arginine (R) in these variable positions. Other common microcystin variants include YR, RR, and LA (Sivonen and Jones 1999). They differ in methyl group and 2 amino acids within the ring, resulting in marked differences in toxicity and in hydrophobic or hydrophilic properties. Microcystins have the general structure cyclo (-D-Ala-L-*X*-erythro-β-methyl-D-isoAsp-L-*Z*-Adda-D-isoGlu-*N*-methyldehydro-Ala), where *X* and *Z* represent positions for L amino acids. The Adda amino acid has become a useful tool in microcystin research because it provides the molecule with a characteristic wavelength absorbance at 238 nm. The absorbance characteristics of Adda provide a means of analyses of microcystins after separating them by reverse-phase high pressure liquid chromatography (HPLC). This is believed to be attributed to the conjugated diene group in the long carbon chain of this uncommon amino acid. The Adda moiety is also required for toxicity and is important in the binding of the toxin to protein phosphatases. The stereochemistry of the dienes of the Adda group has also been shown to influence toxicity and also the levels of methylation of various structures in the cyclic peptide.

Nodularins

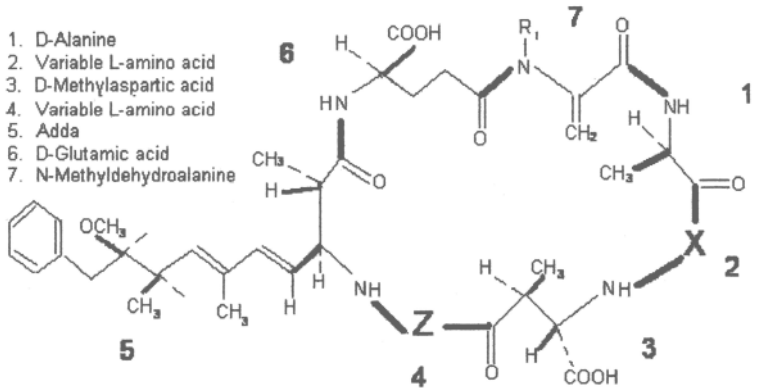
Nodularins, with several structural variants, are found predominantly in marine, brackish waters, which are not usually used as drinking source. A bloom-infested lake in Australia containing *N. spumigena* was shown to be the cause of stock deaths, and the toxin has since been isolated and characterized (Rinehart et al. 1988) as nodularin. The mammalian toxicity of microcystins and nodularins is mediated through their strong binding to protein phosphatases (which are important molecular switches in all eukaryotic cells) through an irreversible bond (MacKintosh et al. 1990). In solution, microcystins and nodularins adopt a chemical "shape" that is similar especially in the Adda-glutamate part of cyanotoxin molecule (Annila et al. 1996).

Table 2. Characteristics of common biotoxins.

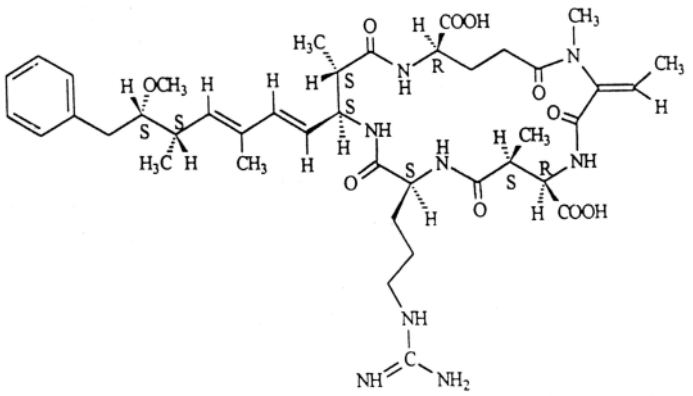
Toxin produced	Type/nature	Mode of action	Cyanobacterial genera	LD_{50}
Microcystin	Hepatotoxin; cyclic peptide	Block protein phosphatases by covalent binding and cause hemorrhaging of the liver; cumulative damage may occur	*Microcystis*, *Anabaena*, *Anabaenopsis*, *Hapalosiphon*, *Nostoc*, *Planktothrix* (*Oscillatoria*)	45≥1000
Microcystin-LR	Hepatotoxin; cyclic peptide	Block protein phosphatases by covalent binding and cause hemorrhaging of the liver; cumulative damage may occur	*Microcystis*, *Anabaena*, *Anabaenopsis*, *Hapalosiphon*, *Nostoc*, *Planktothrix* (*Oscillatoria*)	25–125
Microcystin-YR	Hepatotoxin; cyclic peptide	Block protein phosphatases by covalent binding and cause hemorrhaging of the liver; cumulative damage may occur	*Microcystis*, *Anabaena*, *Anabaenopsis*, *Hapalosiphon*, *Nostoc*, *Planktothrix* (*Oscillatoria*)	70
Microcystin-RR	Hepatotoxin; cyclic peptide	Block protein phosphatases by covalent binding and cause hemorrhaging of the liver; cumulative damage may occur	*Microcystis*, *Anabaena*, *Anabaenopsis*, *Hapalosiphon*, *Nostoc*, *Planktothrix* (*Oscillatoria*)	300–600
Nodularin	Hepatotoxin; pentapeptide	Similar to that of microcystin, with death resulting from haemorrhage into the liver; induces pores in bilayers of diphytanoyl lecithin and locust in muscle membrane	*Nodularia spumigena*	30–50
Anatoxin	Neurotoxin	—	—	—
Anatoxin-a	Neurotoxin; secondary amine	Block postsynaptic depolarization	*Anabaena*, *Oscillatoria*, *Planktothrix*	250
Homoanatoxin-a	—	—	*Oscillatoria formosa*	250
Anatoxin-a(s)	Neurotoxin; organophosphate	Block acetyl cholinesterase	*Anabaena flos-aquae*	40
Saxitoxin	Neurotoxin; carbamate alkaloid	Block neuron sodium channels across the axon membrane	*Planktothrix*, *Anabaena*, *Aphanizomenon*	10–30
Cylindrospermopsin	Hepatotoxin; cylindrospermopsin alkaloid	Block protein synthesis	*Cylindrospermopsis raciborskii*	2100 in 1 day and 200 in 5–6 days
Lipopolysaccharide	Endotoxin; lipopolysaccharide	—	*Microcystis*, *Anabaena*, *Nostoc*, *Anabaenopsis*, *Hapalosiphon*, *Planktothrix*, *Anacystis*, *Aphanizomenon*, *Lyngbya*, *Cylindrospermopsis*, *Nodularia*, *Schizothrix*, *Umezakia*	—

Note: Information was compiled from Turner et al. 1990; Frankmolle et al. 1992; Falch et al. 1993; Kuiper-Googman et al. 1999; Sivonen and Jones 1999; Dow and Swoboda 2000.

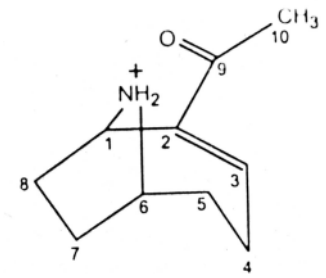
Fig. 1. Structures of commonly known cyanotoxins.



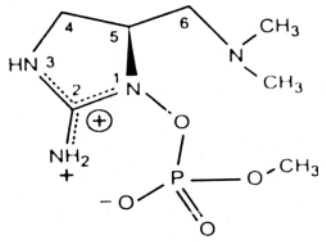
MICROCYSTIN-GENERAL STRUCTURE



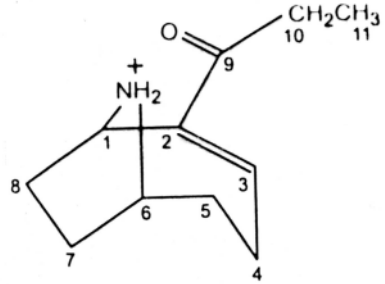
NODULARIN



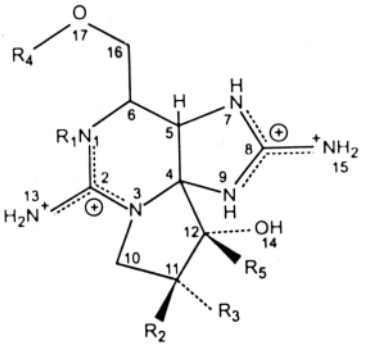
ANATOXIN-A



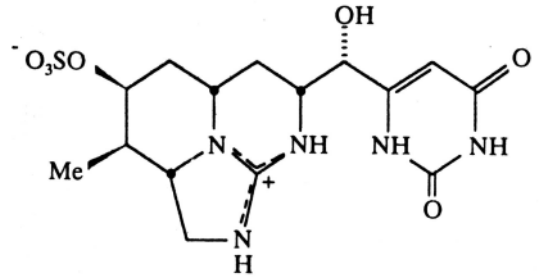
ANATOXIN-A(S)



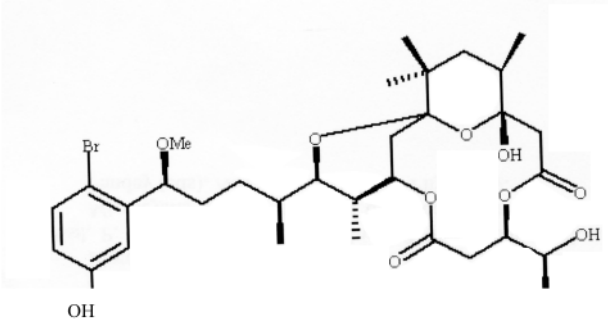
HOMOANATOXIN-A



SAXITOXIN GENERAL STRUCTURE



CYLINDROSPERMOPSIN



DEBROMOAPLYSIATOXIN

Alkaloid toxins

Alkaloids vary in their chemical structure and stabilities, and depending on the type, they can affect the nervous system, skin liver, or gastrointestinal tract. Some of the most studied algal toxins belonging to alkaloid class include anatoxin-a, anatoxin-a(s), homo-anatoxin-a, saxitoxin, and cylindrospermopsin.

Anatoxins

The anatoxins are a group of neurotoxic alkaloids. They are low molecular weight secondary amines produced by a number of cyanobacterial genera, including *Anabaena*, *Oscillatoria*, and *Aphanizomenon*. The toxicity of these compounds (LD_{50}) varies from 20 μg/kg (by mass, intraperitoneally in mice) for anatoxin-a(s) to 200–250 μg/kg for anatoxin-a and homoanatoxin-a, making them more toxic than many microcystins. Anatoxin-a is produced by some strains of *Anabaena*. It is a structural analogue of cocaine and the neurotransmitter acetylcholine and binds to nicotinic acetylcholine receptors. Death occurs by respiratory failure within minutes to few hours depending upon the species (Carmichael 1994; Hunter 1995). Homo-anatoxin-a is a potent neuromuscular blocking agent that causes severe paralysis, convulsions, and death by respiratory arrest. This toxin has been purified from *Oscillatoria formosa*. Anatoxin-a(s) is an organophosphate (Fig. 1) produced by strains of *Anabaena flos-aquae* (Matsunaga et al. 1989). This is the only known naturally occurring organophosphate, which functions as cholinesterase.

Saxitoxins

Like anatoxins, the saxitoxins (STX) are neurotoxic alkaloids, which are also known as PSPs (paralytic shellfish poisons) owing to their occurrence and association with seafood. Saxitoxins and their analogues are produced by *Anabaena* and *Aphanizomenon* (Sawyer et al. 1968). They have also been recorded from *Lyngbya wollei* (Carmichael et al. 1997). They act on the sodium ion channel of the excitable membranes of the nerve cells and block them, thus causing their neurotoxic effects like paralysis or respiratory failure (Hunter 1995). However, to date no PSP-like illness has been reported in humans from consumption of drinking water. There are a number of STX variants generally divided into groups based on their structure or organism of origin. The single sulphated STXs are known as gonyautoxins and the doubly sulphated STXs are known as C-toxins. There are also decarbamyl STXs (dcSTX) and a group of STX variants, so far found only in *L. wollei*, known as *Lyngbya-wollei*-toxins. STXs are highly toxic with LD_{50}s as low as 10 μg/kg (intraperitoneally in mice). (See Fig. 1 for the general structure of STX.)

Cylindrospermopsin

Cylindrospermopsin is a hepatotoxic alkaloid (Fig. 1) that has been isolated from cultures of *Cylindrospermopsis raciborskii* (Ohtani et al. 1992). It blocks protein synthesis, the first clinical symptoms being kidney and liver failure. In contrast to pure toxin, the crude extract of the organism also causes injury to lungs, adrenal glands, and intestines, indicating the presence of further unknown toxin in the organism. This toxin has been identified in an outbreak of acute hepato-enteritis and renal damage among an aboriginal population in Australia (Falconer 1996). Cylindrospermopsin has also been characterized from *Aphanizomenon ovalisporum* in Israel (Banker et al. 1997) and *Umezakia natans* in Japan (Harada et al. 1994). Its LD_{50} (intraperitoneally in mice) of 200 μg/kg ranks it as a relatively potent cyanobacterial toxin.

LPS toxins (endotoxin)

Weise and co-workers (1970) were the first to isolate LPS from the cyanobacterium *Anacystis nidulans*, which exhibits both pyrogenic and toxic effects (Weckesser et al. 1979). But unlike Gram-negative bacteria, cyanobacterial LPS lacks any phosphate in the lipid A core (Keletis and Sykora 1982). Cyanobacterial LPSs are found to be 10 times less toxic than other bacterial LPSs, e.g., those produced by *Salmonella* sp. (Codd 1984), and reports of human illness due to these toxins exist (Codd and Poon 1988). There is considerable diversity among the cyanobacteria in the chemical composition; however, the differences are largely related to phylogeny.

Cytotoxins

Although neurotoxins and hepatotoxins are more studied due to their lethality and ubiquity, cytotoxins are equally important due to their antialgal, antimycotic, and antibacterial activity and some are active against cell tissue lines and have moderate antitumor activity (Gerwick et al. 1994; Patterson et al. 1994). Some common cyanotoxins, their mode of action, their structure, and the organism producing them are listed in Table 2.

Cytotoxins are interesting compounds because they are more targeted and can be exploited as antitumor, antiviral, or antibiotic compounds (Gross 2003). In general most cytotoxins have a strong cytostatic activity or an ability to selectively inhibit tumors. However, they are highly heterogeneous in their chemical structure and in terms of the qualitative and quantitative aspects of damage inflicted. The majority of them are cyclic or aliphatic peptides with protease inhibition activity, e.g., micropeptins, cyanopeptides, cyanopeptolins, all of which have been isolated from several planktonic species belonging to diverse colonial and filamentous genera — *Microcystis*, *Anabaena*, and *Planktothrix* (Moore 1996; Namikoshi and Rinehart 1996). Scytophycins represent another well-investigated group of cytotoxins that have been mostly isolated from branched cyanobacteria, e.g., *Scytonema*, *Tolypothrix*, and *Cylindrospermum muscicola* (Jung et al. 1991). Among this group of macrolytic lactones, tolytoxin has been investigated in depth. It causes severe cytotoxic effect through the depolarization of cell microfilaments (Patterson and Carmeli 1992). Another group of promising compounds are the cryptophycins, which are lipophilic compounds mainly isolated from *Nostoc* strains (Trimurtulu et al. 1994). The cytotoxic effects of methanolic extracts belonging to several soil isolates of the genera *Anabaena*, *Nodularia*, *Cylindrospermum*, *Tolypothrix*, and *Trichormus* have also been shown on mammalian cell lines (Hrouzek et al. 2005). Similar studies with microcystins have shown that these compounds are capable of initiating apoptosis in hapatocytes (Botha et al. 2004), besides their well-investigated effects on increased protein phosphorylation due to protein phosphatase inhibition of mammalian cell lines (Carmichael 1994).

Table 3. Characteristics of common cytotoxins.

Toxin produced	Type/nature	Mode and (or) site of action	Cyanobacterial genera	Reference
Acutiphycin	Phospholipid compound	Antitumor properties, affects tissues	*Oscillatoria acutissima*	Barchi et al. 1984
Ambigol A and B	Polychlorinated aromatic compound	Inhibits cyclooxygenase and HIV reverse transcriptase	*Fischerella ambigua*	Falch et al. 1993
Aplysiatoxin	Dermatotoxic	Induces Limulus amoebocyte	*Lyngbya*	Dow and Swoboda 2000
Calothrixin	Indolophenanthridine	Inhibits RNA synthesis and DNA replication	*Calothrix* sp.	Doan et al. 2000
Cryptophycins A–F	Lipophilic peptides	Selectively inhibits tumors	*Nostoc* spp.	Trimurtulu et al. 1994
Cyanobacterin	Lipophilic aromatic compound	Inhibits photosystem II activity	*Scytonema hofmannii*, *Nostoc linckia*	Gleason and Case 1986
Debromoaplysiatoxin	Dermatotoxic	Lysate gelation	*Schizothrix calcicola*, *Oscillatoria nigroviridis*	Dow and Swoboda 2000
Fischerellin	Aminoacylpolyketide	Inhibits photosystem II activity	*Fischerella ambigua*, *Fischerella muscicola*	Gross et al. 1991; Papke et al. 1997
Grassypeptolide	Macrocyclic depsipeptide	Inhibits cancer cell growth	*Lyngbya confervoides*	Kwan et al. 2008
Hapalindole	Substituted indole alkaloid	Inhibits tumors	*Hapalosiphon fontinalis*, *Fischerella* sp.	Moore et al. 1984; Doan et al. 2000; Etchegaray et al. 2004; Asthana et al. 2006*b*
Hassallidin A	Glycosylated lipopeptide	—	*Hassillia* sp.	Neuhof et al. 2005
Laxaphycin A and B	Cyclic peptide	Inhibits tumor cell proliferation	*Anabaena laxa*	Frankmolle et al. 1992
Linolenic acid	Polyunsaturated fatty acid	Decreases blood cholesterol levels, reduces the growth of cancers of the breast and colon	*Oscillatoria redekei*, *Fischerella* sp.	Mundt et al. 2001; Asthana et al. 2006*a*
Lyngbyatoxin	Indole alkaloid	Potent tumor promoter, activates calcium-activated phospholipid-dependent protein kinase C	*Lyngbya majuscula*	Cardellina et al. 1979; Fujiki et al. 1983; Nishizuka 1984
Norharmane	Indole alkaloid	Inhibits indoleamine 2,3- dioxygenase and nitric oxide synthase	*Nodularia harveyana*	Volk 2005
Nostocine	—	Generates toxic reactive oxygen species	*Nostoc spongiaeforme*	Hirata et al. 2003
Nostocyclamide	Cyclic heptapeptide	Inhibits photosynthesis	*Nostoc* sp. strain 31	Juttner et al. 2001
Oscillatorin	Lipophilic compound	Inhibits photosystem II activity	*Oscillatoria latevirens*	Bagchi 1995; Ray and Bagchi 2001
Scytophycin	Lipophilic compound	Depolarizes cell microfilaments	*Scytonema pseudohofmanni*, *Tolypothrix*, *Cylindrospermum muscicola*	Moore et al. 1986; Carmichael et al. 1990; Jung et al. 1991
Spiroidesin	Lipopeptide	Inhibits cell growth	*Anabaena spiroides*	Kaya et al. 2002

The cytotoxins that have been isolated from cyanobacteria are listed in Table 3 along with their major characteristics and modes of action.

Detection techniques for cyanotoxins

The physical appearance of cyanobacterial bloom does not reveal its toxicity, and diagnosis of cyanotoxicosis is difficult primarily because many blooms are not hazardous at all times.

The development of more sensitive separation and detection methods will help in reducing the consequences of these poisons in our food and water supply. A range of other assay procedures have been investigated, which range from low cost and relatively simple methods to highly sensitive and sophisticated methods. Detection of toxin depends upon the type of information required and facilities available.

Toxicity tests and bioassay

Mouse bioassay

There have been many biological detection methods developed for detecting cyanotoxins that use bioactivity of toxins, e.g., potent hepatotoxicity, neurotoxicity, cytotoxicity enzymatic activity, and immunological interactions. Mouse bioassay was typically the first test for toxicity used in screening water bloom materials and laboratory cultures or cell extracts. It is also considered as the standard procedure for establishing the LD_{50}. It is still preferred over other methods because of the easy availability of laboratory mice and the low cost. In this method, adult mice are injected intraperitoneally with the sample to be assayed and are kept under observation. Generally, toxic symptoms are visible within 24 h. This method is able to detect the toxin within minutes (to a few hours) and can possibly determine the type of toxin present, i.e., hepatotoxin, neurotoxin, etc. The disadvantages of this method are the nonspecific inference and (or) result obtained, its inability to detect low levels of toxin, and its inability to distinguish between homologues of toxins. Mouse bioassay remains the primary means of assessing function and potency of cyanotoxins, but this is now slowly being replaced by other bioassays — chemical and immunological methods. Further, there is increasing opposition in many countries to the use of animals for any form of toxicity testing.

Alternative bioassay

A number of alternative bioassay methods have been devised for detection of cyanotoxins, e.g., by using *Daphnia* sp. and *Artemia salina* (Kiviranta et al. 1991). Turell and Middlebrook (1988) suggested a bioassay that involved microinjection of the toxin into mosquitoes. The use of both adult and larvae stages has been investigated as potential bioassay agents. Both methods are relatively simple but not have been widely adopted owing to difficulties in handling this organism (Turell and Middlebrook 1988; Kiviranta et al. 1991). The other test organism, which can detect microcystins successfully in the bloom samples, is the fruit fly *Drosophila melanogaster* (Swoboda and Dow 1994). These organisms are easy to maintain in the laboratory with no special equipment required. The flies were, however, not sensitive to neurotoxic *Aphanizomenon* (Swoboda and Dow 1994). McElhiney et al. (1998) reported that the locust bioassay was able to detect saxitoxin successfully in a range of samples that included cyanobacteria and shellfish, but the bioassay was not found sensitive to microcystin-LR and anatoxin-a.

Biochemical assays

The protein phosphatase inhibition assay is a sensitive screening method for microcystin and nodularin, which uses the biochemical activity of these enzymes. One version is based on the quantification of ^{32}P-phosphate released from radiolabelled substrate (Lambert et al. 1994) by the activity of protein phosphatase enzyme (PP1 and PP2). Although this method is very popular, the only disadvantage is the use of radioactive chemicals, which requires specialized and well-equipped laboratory facilities. Carmichael (1994) used a colorimetric protein phosphatase inhibition assay, which avoids the complications of using radioactive materials. Ward et al. (1997) also reported this method is an effective and reliable means of screening water samples.

Immunological detection

The Enzyme-Linked Immunosorbant Assay (ELISA) technique is currently the most promising for rapid sample screening of microcystins owing to its sensitivity, specificity, and ease of operation. Chu et al. (1989) developed an ELISA technique based on polyclonal antisera in rabbit against bovine albumin conjugated to microcystin-LR, which proved to be a better immunogen. The raised antibodies had good cross-reactivities with microcystin-RR, -YR, and nodularin and less with microcystin-LY and -LA. Approximately 50% binding occurred at a toxin concentration of 1 ng/mL, which is appropriate for water quality testing. Nagata et al. (1995) produced monoclonal antibodies against microcystin-LR, which showed cross-reactivity with microcystin-RR, -YR, -LA, and several other derivatives. Although the epitope of this antibody is not clear, the importance of the Adda moiety for antibody binding has been indicated.

Analytical methods

The physicochemical properties of the cyanotoxins can be used for detection and identification. The molecular weight, chromophores, and reactivities of the functional groups in the molecules are generally used for the chemical analyses of the cyanotoxins. The majority of analytical methods have been developed primarily for microcystins but nodularins can also be easily identified by the same method because of the similar physicochemical properties.

Thin layer chromatography (TLC) is the most basic technique that has been used to purify hepatotoxins, but it is not very sensitive and specific; hence, over time, it has been superceded by HPLC. Typical HPLC analysis uses a reverse-phase C18 silica column, and separation is achieved over a gradient of acetonitrile and water, both containing 0.05% trifluoroacetic acid. However, this method relies on retention time for identification and microcystin standards are required (Harada 1996). Recent advances in detector hardware can now provide high-resolution spectra that detect very slight variations in chemical composition and can be used in conjunction with advanced spectral matching

software. Further confirmation may be done by liquid chromatography – mass spectrometry, which enables simultaneous separation and identification of microcystins from a mixture of samples (Kondo et al. 1992). A more advanced technique — frit-fast atom bombardment liquid chromatography – mass spectrometry (which uses a microbore column (0.3 mm internal diameter)) — can identify nanogram levels in water and biological samples (Kondo et al. 1995, 1996). The structural analyses of hepatotoxins can be carried out by fast atom bombardment mass spectrometry and proton nuclear magnetic resonance (Kusumi 1996). Recently, matrix-assisted laser desorption ionization mass spectrometry has shown to be a rapid and sensitive analytical method for the detection of cyanobacterial toxins (Dow et al. 1994).

Molecular detection

Traditionally, microscopic identification of cyanobacteria alone or in combination with direct analysis of toxin has been used for detection of toxic cyanobacteria. However, some cyanobacteria may be genetically capable of producing toxins but do not produce under all conditions and some do not produce toxins all. Hence, scientists are focusing on genes that can differentiate taxonomic groups of cyanobacteria involved in toxin production and those that are not toxic, i.e., genes that can separate toxic and nontoxic strains (Neilan et al. 1995; Bloch et al. 1996; Rouhianen et al. 2004). Bloch et al. (1996) have looked for conserved regions of genome, such as those encoding for phycocyanin subunits, that might be used to classify the toxigenic species. Neilan et al. (1995) designed a general primer to the phycocyanin operon (*cpc* genes) and developed a PCR that allowed amplification of the pycocyanin (PC) gene containing the intergenic spacer from cyanobacteria commonly associated with toxic bloom sample. They showed that the DNA profile obtained after restriction digestion with 9 enzymes was specific to various taxonomic levels of cyanobacteria. This kind of gene probe, which can distinguish among toxic and nontoxic genera and (or) strains, can prove to be very beneficial in detecting toxic cyanobacterial bloom or screening cyanobacteria for toxicity. Neilan et al. (1995), using restriction length polymorphism, probed a short tandemly repeated DNA sequence found in the chromosome of *Anabaena* sp. PCC 7120 and found that it can distinguish a hepatotoxic *Anabaena* isolate from neurotoxin-producing strains. Baker et al. (2001) showed from their studies that bloom component can be identified and monitored for toxigenicity by PCR more effectively than by other methods, like microscopy and mouse bioassay. They used PCR amplification of a phycocyanin intergenic spacer region between the genes for β and α subunits for detection of cyanobacterial genera. Microcystin production was determined by PCR amplification of gene of microcystin biosynthesis and the potential for saxitoxin production was determined by PCR amplification of a region of 16S rRNA gene of *Anabaena circinalis*. They also showed that toxicity and cell type present in a bloom changes over a period of time. Fergusson and Saint (2000) developed an *Anabaena circinalis*-specific PCR assay targeting the *rpoCI* gene to detect the organism directly from environmental samples. Wilson et al. (2000) developed a PCR assay targeting a region of *rpoCI* gene unique to *Cylindrospermopsis raciborskii* for specific identification of the strain from both purified genomic DNA and environmental samples.

Genetic basis of toxin production

At present, 2 molecular systems are known to be involved in cyanobacterial toxicity: nonribosomal peptides and Type I polyketide synthases. Most of the work involving this aspect of toxin production has been done using *Microcystis* (Tillett et al. 2001; Ouellette et al. 2006), and the expression of these genes is known to be regulated by complex mechanisms and is influenced by environmental factors.

Biosynthesis of microcystins has been studied in all the 3 main microcystin-producing genera occurring in fresh water, i.e., *Microcystis*, *Anabaena*, and *Planktothrix* (Tillett et al. 2001; Christiansen et al. 2003, 2006; Rouhianen et al. 2004). Microcystins are synthesized nonribosomally by a giant enzyme complex comprising peptide synthetase, polyketide synthases (PKSs), and additional modifying enzymes (Arment and Carmichael 1996; Tillett et al. 2001; Christiansen et al. 2003; Rouhianen et al. 2004). Nonribosomal peptide synthetases catalyse the formation of peptide by a thio-template mechanism. They are involved in the synthesis of linear, cyclic, and branched cyclic peptides, including potent drugs such as penicillin, vancomycin, and cyclosporin (Konz and Marahiel 1999).

The gene cluster encoding the microcystin synthetase complex has now been identified and sequenced (Nishizawa et al. 1999; Tillett et al. 2001). This 55-kb gene cluster (*mcyABCDEFGHIJ*) consists of 6 open reading frames (ORFs) of a mixed nonribosomal peptide synthetase – polyketide synthase nature (*mcyA–mcyE* and *mcyG*) and 4 smaller ORFs with putative precursor and tailoring functions (*mcyF* and *mcyH–mcyJ*). The *mcyABCDEFGHIJ* genes are transcribed as 2 polycistronic operons, *mcyABC* and *mcyDEFGHIJ*, from a central bi-directional promoter between *mcyA* and *mcyD*. Kaebernick et al. (2002) were able to identify the start sites for all the *mcy* genes except for *mcyB* and *mcyC*. Baker et al. (2002) also suggested the use of PCR-based methods for direct detection and identification of strains present and their toxigenicity. Vaitomaa et al. (2003) used quantitative RT–PCR analyses to show that *mcyE* gene copy numbers can be used as surrogates for a rough estimate of toxigenic cell numbers of hepatotoxic *Microcystis* and *Anabaena* spp. This can provide a simple method for detecting toxic blooms in diverse areas and (or) in a large number of samples. Toxic *Microcystis* strains often produce several isoforms of cyclic hepatotoxin microcystin that have been attributed to relaxed substrate specificity and adenylation domains. Mikalsen et al. (2003) showed that this variability was also caused by genetic variation in a microcystin synthetase gene. Moffitt and Neilan (2004) sequenced and characterized a complete gene cluster encoding the enzymatic machinery required for the biosynthesis of nodularin in *N. spumigena* NSOR 10. Kaebernick et al. (2000) studied the effect of light on microcystin synthetase production and concluded that both *mcyB* and *mcyD* transcript level increased under high light intensities and red light, blue light and certain artificial stress factors (methylogen and NaCl) caused reduction in transcript

levels. Tonk et al. (2005) also reported that microcystin composition of the cyanobacterium *Planktothrix agardhii* changes to a more toxic variant with increasing light intensity. Kurmayer et al. (2004) reported the occurrence of *Planktothrix* strains containing all *mcy* genes but lacking toxic hepatopeptide microcystin. Christiansen et al. (2006) analyzed 29 strains and showed that transposons inactivate biosynthesis of nonribosomal peptide microcystin. Two deletions spanning 400 bp (in *mcyB*; one strain) and 1869 bp (in *mcyHA*; 3 strains) and 3 insertions spanning 1429 bp (in *mcyD*; 8 strains), 1433 bp (in *mcyEG*; one strain), and 1433 bp (in *mcyA*; one strain) were identified, indicating that a mutation resulted in the inactivation of microcystin biosynthesis. Transcriptional analyses of the *mcy* gene cluster will help to increase our understanding of microcystin synthetase regulation and toxin biosynthesis and will provide useful insight into other nonribosomal systems.

Ecological implications of cyanobacterial secondary metabolites

Cyanobacteria represent an untapped bioresource for a diverse range of secondary metabolites, some of which show unique similarities to plant and animal products (Namikoshi and Rinehart 1996; Bagchi and Ray 2001; Agrawal et al. 2005; Singh et al. 2005*a*; Capper et al. 2006; Dahms et al. 2006; Volk 2006). Toxins produced by bacteria, algae, and (or) cyanobacteria can be considered allelochemicals, since they inhibit one or more groups of organisms and help to provide a competitive advantage to the producer, which may be important factors contributing to formation and (or) maintenance of cyanobacterial blooms (Gross 2003; Legrand et al. 2003; Prasanna and Jaiswal 2006; Leflaive and Ten-Hage 2007).

Allelochemicals

The term allelopathy is derived from 2 separate words (*allelon*, which means "of each other," and *pathos*, which means "to suffer"), and it denotes the production of specific biomolecules by one plant or bacterial species that can induce suffering in, or give benefit to, another plant or bacterial species. In simple terms, allelopathy refers to the chemical inhibition of one species by another. These biomolecules, or allelochemicals, produced by one species are released into the environment and subsequently influence the growth and development of neighbouring species. Allelochemicals in general have evolved to inhibit many biochemical targets present on the surface of or within the cells (Skovgaard et al. 2003; Suikkanen et al. 2005). Many such chemicals, including antibiotics, inhibit components of protein synthesis. Both positive and negative allelochemical incidents are known to be involved in the control of freshwater bloom sequence (Keating 1977; Gross 1999). Schlegel et al. (1999) isolated cyanobacterial strains form diverse habitats, spanning the continents of Australia and Asia, that exhibited allelopathic activity against green algae and cyanobacteria. Gross (1999) reported the production of allelochemicals with a strong effect against several algae (including cyanobacteria). Jaiswal et al. (2005) reported allelopathic activity of *M. aeruginosa* against a unicellular cyanobacterium. The colonial blue-green alga *Merismopedia tenuissima* has been reported (Blomquist 1996) to dominate an acidic Swedish lake due to the release of allelochemicals. Hence, the prolific growth of cyanobacteria as blooms in nutrient-rich water bodies, concomitant with eutrophication, can be considered a result of their invasiveness and production of bioactive molecules, which provides them the advantage of eliminating other competitive flora and fauna. This represents an appropriate example of how allelopathy, nutrient mobilization, and resource competition act synergistically in providing a selective advantage to these prokaryotes.

Aquatic allelochemicals often target multiple physiological processes. A filamentous cyanobacterium, *Scytonema hofmannii* (UTEX 2349), has been reported to produce an allelochemical cyanobacterin, which inhibited the growth of cyanobacteria (Gleason and Paulson 1984), eukaryotic algae (Gleason and Baxa 1986), and higher plants (Gleason and Case 1986). Non-photosynthetic microorganisms were not affected by the cyanobacterin. Similar observations were made in *Oscillatoria* (Bagchi et al. 1990; Chauhan et al. 1992) and *Fischerella* (Gross et al. 1991), *Nostoc* (Juttner et al. 2001), where allelochemicals released by the cyanobacteria inhibited growth of cyanobacteria and chlorophytes but not heterotrophic organisms. The most common mode of action of these metabolites is directed towards the inhibition of photosynthesis by affecting photosystem II activity (Bagchi et al. 1990; Bagchi 1995; Srivastava et al. 1998, 2001). Smith and Doan (1999) also reported that bioactive compounds produced by cyanobacteria (allelochemicals) show a diverse range of biological activities and affect many biochemical processes within the cell, especially the oxygenic photosynthetic processes. Such chemicals are likely to be involved in the regulation of natural populations and are potentially useful as biochemical tools, i.e., as herbicidal and biocidal agents. Bagchi and Ray (2001) reported that a planktonic cyanobacterial extract showed photosystem II-inhibiting (herbicidal) activity, besides allelopathic activity, which might play a role in competitive ability of the strain in nature. Doan et al. (2000) reported alkaloids from *Fischerella* and *Calothrix* that inhibited RNA synthesis of other organisms, indicating another mode of action of cyanobacterial secondary metabolites. A bloom-forming cyanobacterium *Anabaena flos-aquae* has been reported to be involved in chemical signaling with the competing phytoplankton. This cyanobacterium produces anatoxin and microcystin-LR, which inhibit the growth of chlorophyte *Chlamydomonas reinhardtii*, whereas the extracellular compound produced by the chlorophyte causes an increase in anatoxin content but no effect on microcystin-LR. (Kearns and Hunter 2001). The exudates from *Microcystis* have also been reported to inhibit the photosynthesis in the dinoflagellate *Peridium gatunnse* by interfering with its internal carbonic anhydrase activity (Sukenik et al. 2002). However, inhibition was reported in a strain that did not produce microcystin-LR, which is indicative of the role of some other compound. The compound released by the *Microcystis* sp. resulted in oxidative stress in the dinoflagellate and activated certain protein kinases (Vardi et al. 2002), while *P. gatunnse* caused cell lysis in *Microcystis* owing to a loss of buoyancy and a dramatic increase in *mcyB*. These 2 organisms present excellent examples of complex allelopathic interactions.

Multiple biotic and abiotic factors determine the strength of allelopathic interaction. Frequently, the impact of excessive or limiting nutrients has been shown to affect the overall production of allelochemicals and their effect on target organisms (Von Elert and Juttner 1997; Ray and Bagchi 2001; Gross 2003; Hirata et al. 2003; Volk 2007). Jaiswal et al. (2007) showed that doubling the concentration of phosphorus in the basal medium resulted in increased growth and algicidal activity (measured in the form of diameter of inhibition zone) of *M. aeruginosa.* However, no activity was recorded when phosphate concentration was reduced to half (50% of that present in the basal medium). In the same study, they also reported that in *M. aeruginosa* cultures at all stages showed algicidal activity; however, maximum inhibitory action was recorded in 15- to 20-day-old cultures.

Suikkanen et al. (2004) found that *N. spumigena* was more allelopathic in exponential phase than in stationary phase, whereas the culture filtrates were more hepatotoxic in stationary phase.

Bioactive compounds

Cyanobacteria have also drawn much attention as a prospective and rich bioresource of biologically active compounds that can be agriculturally useful. Toxic water blooms comprising genera, such as *Microcystis*, *Anabaena*, and *Nostoc*, produce a diverse array of bioactive compounds exhibiting antibiotic, algicidal, antifungal, cytotoxic, immuno-suppressive, and enzyme-inhibiting activities (Mundt et al. 2001). Terrestrial cyanobacteria, such as *Anabaena laxa*, produce antifungal cyclic peptides, such as laxaphycin A and B (Frankmolle et al. 1992). Kulik (1995) reported that extracts from the cyanobacterium *Nostoc muscorum* Agardh inhibited in vitro growth of fungal plant pathogens, such as *Sclerotinia sclerotiorum* (Cottony rot of vegetables and flowers) and *Rhizoctonia solani* (root and stem rots). Another antifungal glycosylated lipopeptide has been reported (Neuhof et al. 2005) from the cyanobacterium *Tolypothrix* (Basionym *Hassallia*) that showed antifungal activity against *Aspergillus fumigatus* and *Candida albicans*. The antifungal compound had been reported to contain both fatty acid and carbohydrate moieties. A number of compounds exhibiting fungicidal activity against specific agriculturally important fungi have been isolated and patented (Moore et al. 1989; Patterson et al. 1995; Hagmann and Juttner 1996; Moore 1996; Nagle and Wedge 2002; Volk and Furkert 2006). Pushparaj et al. (2000) showed that an acetone extract of *Nodularia harveyana* inhibited growth of the free-living root knot nematode *Cephaloboides oxycerca*. Such biomolecules produced by cyanobacteria can be developed into safe formulations against many pathogen and (or) parasites.

The terrestrial cyanophyte *Fischerella ambigua* has been reported (Falch et al. 1993) to produce 2 highly halogenated compounds with antibacterial, antifungal, and molluscicidal activity. Kiviratna and Abdel-Hameed (1994) reported a compound from the cyanobacterium *Oscillatoria agardhii* that is toxic to larvae of yellow fever mosquito *Aedu aegypti*. Casamatta and Wickstrom (2000) observed that the exudates of *M. aeruginosa* were inhibitory towards bacterial plankton communities. Recently Asthana et al. (2006*a*) reported antibacterial activity of a methanolic extract of a cyanobacterium *Fischerella* sp. that was further identified as linolinic acid (Asthana et al. 2006*b*). Antimicrobial and other bioactive compounds produced by cyanobacteria may be released extracellularly to surroundings either actively or passively or by autolysis. The biosynthetic pathways leading to production of these novel compounds and finally their release to the environment is not very well understood. Knowledge of biosynthetic pathways and factors leading to the release of these biomolecules may prove to be of tremendous importance for mankind.

The chemical compounds isolated from cyanobacteria are also of biotechnological interest, especially for clinical applications, because of their antibiotic, algicidal, and cytotoxic properties (Borowitzka 1995) and, hence, could be used as biocontrol agents of bacterial and fungal pathogens. In addition to neurotoxins and hepatotoxins, cyanobacteria are known to produce several antibacterial compounds (Carmeli et al. 1990), antifungal compounds (Frankmolle et al. 1992), antiviral agents, anticancerous and (or) antineoplastic agents (Smith et al. 1994; Nianjun et al. 2004), and compounds useful in the treatment of HIV (Gerwick et al. 1994; Harrigan et al. 1998). Cyanobacterial metabolites have the potential for use in antifouling technology. The isolation of biogenic compounds and the determination of their structure may provide leads for future development of environmentally friendly antifouling paints (Dahms et al. 2006).

Cyanobacteria represent a vast bioresource of biologically active compounds that may find tremendous applications in agriculture and pharmaceutical industry. So far, only a few strains have been commercially exploited, and extensive research is needed to unfold many unknown facets hidden inside these small microorganisms, which through their omnipresence and adaptation to diverse environment, can be exploited for diverse applications.

Acknowledgements

The authors are thankful to the Council of Scientific and Industrial Research (CSIR) and the Indian Council of Agricultural Research, New Delhi, India, for providing financial assistance, in the form of projects. The facilities required for carrying out this investigation provided by the Centre for Conservation and Utilization of Blue-Green Algae, IARI, New Delhi, are gratefully acknowledged.

References

Agrawal, M.K., Bagchi, D., and Bagchi, S.N. 2005. Cysteine and serine protease-mediated proteolysis in body homogenate of a zooplankter, *Moina macrocopa*, is inhibited by the toxic cyanobacterium, *Microcystis aeruginosa* PCC7806. Comp. Biochem. Physiol. Biochem. Mol. Biol. **141**: 33–41. doi:10.1016/j.cbpc.2005.01.002.

Annila, A., Lehtimaki, J., Maltila, K., Eriksson, J.E., Sivonen, K., Rantala, T.T., and Brakenbery, J. 1996. Solution structure of nodularin — an inhibitor of serine/threonine specific protein phosphatases. J. Biol. Chem. **271**: 16695–16702. PMID: 8663277.

Arment, A.R., and Carmichael, W.W. 1996. Evidence that microcysin is a thiol template product. J. Phycol. **32**: 591–597. doi:10.1111/j.0022-3646.1996.00591.x.

Asthana, R.K., Srivastava, A., Kayastha, A.M., Nath, G., and

Singh, S.P. 2006*a*. Antibacterial potential of γ-linolinic acid from *Fischerella* sp. colonizing neem bark. J. Appl. Phycol. **22**: 443–448.

Asthana, R.K., Srivastava, A., Singh, A.P., and Singh, S.P. 2006*b*. Identification of antimicrobial entity from the cyanobacterium *Fischerella* sp. isolated from bark of *Azadiracta indica* (Neem) tree. World J. Microbiol. Biotechnol. **18**: 33–39.

Babica, P., Blaha, L., and Marsalek, B. 2006. Exploring the role of microcystins — a review of effects on photoautotrophic organisms. J. Phycol. **42**: 9–20. doi:10.1111/j.1529-8817.2006.00176.x.

Bagchi, S.N. 1995. Structure and site of action of an algicide from a cyanobacterium, *Oscillatoria late-virens*. J. Appl. Phycol. **10**: 1–9.

Bagchi, S.N., and Ray, S. 2001. Extraction and purification of an algicidal metabolites from a cyanobacterium *Oscillatoria latevirens.* Indian J. Microbiol. **41**: 163–167.

Bagchi, S.N., Palod, A., and Chauhan, V.S. 1990. Algicidal properties of a bloom forming alga, *Oscillatoria* sp. J. Basic Microbiol. **30**: 21–29. doi:10.1002/jobm.3620300106.

Baker, J.A., Neilan, B.A., Entsch, B., and McKay, D.B. 2001. Identification of cyanobacteria and their toxigenicity in environmental samples by rapid molecular analysis. Environ. Toxicol. **16**: 472–482. doi:10.1002/tox.10010. PMID:11769244.

Baker, J.A., Entsch, B., Neilan, B.A., and Mckay, D.B. 2002. Monitoring changing toxigenicity of a cyanobacterial bloom by molecular methods. Appl. Environ. Microbiol. **68**: 6070–6076. doi:10.1128/AEM.68.12.6070-6076.2002. PMID:12450830.

Banker, P.D., Carmeli, S., Hadas, O., Teisch, B., Porat, R., and Sukenik, A. 1997. Identification of cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophyceae) isolated from lake Kinneret. Isr. J. Phycol. **33**: 613–616.

Barchi, J.J., Jr., Moore, R.E., and Patterson, G.M.L. 1984. Acutiphycin and 20,21-didehydroacutiphycin, new antineoplastic agents from the cyanophyte *Oscillatoria acutissima.* J. Am. Chem. Soc. **106**: 8193–8197. doi:10.1021/ja00338a031.

Bloch, C.J.S., Blackburn, S.I., Neilan, B.A., and Grewe, P.M. 1996. Genetic characterization of strains of cyanobacteria using PCR–RFLP of the *cpcBA* intergenic spacer and flanking regions. J. Phycol. **32**: 445–451.

Blomquist, P. 1996. Late summer phytoplankton responses to experimental manipulations of nutrients and grazing in unlimed and limed lake Njupfatet, central Sweden. Arch. Hydrobiol. **137**: 425–455.

Borowitzka, M.A. 1995. Microalgae as a source of pharmaceuticals and other biologically active compounds. J. Appl. Phycol. **7**: 3–15. doi:10.1007/BF00003544.

Botha, N., Gehringer, M.M., Downing, T.G., Venter, M., and Shephard, E.G. 2004. The role of microcystin-LR in the induction of apoptosis and oxidative stress in a CaCo2 cells. Toxicon, **43**: 85–92. doi:10.1016/j.toxicon.2003.10.025. PMID:15037033.

Burja, A.M., Banaigs, B., Abou-Mansour, E., Burgess, J.G., and Wright, P.C. 2001. Marine cyanobacteria — a prolific source of natural products. Tetrahedron, **57**: 9347–9377. doi:10.1016/S0040-4020(01)00931-0.

Capper, A., Cruz-Rivera, E., Paul, V.J., and Tibbetts, I.R. 2006. Chemical deterrence of a marine cyanobacterium against sympatric and non-sympatric consumers. Hydrobiologia, **553**: 319–326. doi:10.1007/s10750-005-1129-x.

Cardellina, J.A., II, Marner, F.J., and Moore, R.E. 1979. Seaweed dermatitis: structure of lyngbyatoxin A. Science, **204**: 193–195. doi:10.1126/science.107586. PMID:107586.

Carmeli, S., Moore, R.E., and Patterson, G.M.L. 1990. Tolytoxin and new scytophycins from three species of *Scytonema.* J. Nat. Prod. **53**: 1533–1542. doi:10.1021/np50072a021. PMID:2128517.

Carmichael, W.W. 1994. The toxins of cyanobacteria. Sci. Am. **270**: 78–86. PMID:8284661.

Carmichael, W.W., Mahmood, N.A., and Hyde, E.G. 1990. Natural toxins from cyanobacteria (blue green algae). *In* Marine toxins, origin, structure and molecular pharmacology. Vol. 418. *Edited by* S. Hall and G. Strichaetz. American Chemical Society, Washington, DC. pp. 87–106.

Carmichael, W.W., Evans, W.R., Yin, Q.Q., Bell, P., and Mocauklowski, E. 1997. Evidence for paralytic shellfish poison in the freshwater cyanobacterium *Lyngbya wollei*. (Farlow ex Gomont) comb. nov. Appl. Environ. Microbiol. **63**: 3104–3110. PMID:9251196.

Casamatta, D.A., and Wickstrom, C.E. 2000. Sensitivity of two disjunct bacterioplankton communities to exudates from the cyanobacterium *Microcystis aeruginosa.* Microb. Ecol. **41**: 64–73.

Chauhan, V.S., Marwah, J.B., and Bagchi, S.N. 1992. Effect of antibiotic from *Oscillatoria* sp. on phytoplankton, higher plants and mice. New Phytol. **120**: 251–257. doi:10.1111/j.1469-8137.1992.tb05661.x.

Christiansen, G., Fastner, J., Erhand, M., Borner, T., and Dittmann, E. 2003. Microcystin biosynthesis in *Planktothrix* genes, evolution and manipulation. J. Bacteriol. **185**: 564–572. doi:10.1128/JB.185.2.564-572.2003. PMID:12511503.

Christiansen, G., Kurmayer, R., Liu, Q., and Borner, T. 2006. Transposon inactivate biosynthesis of non-ribosomal peptide microcystin in naturally occurring *Planktothrix* spp. Appl. Environ. Microbiol. **72**: 117–123. doi:10.1128/AEM.72.1.117-123.2006. PMID:16391033.

Chu, F.S., Huang, X., Wei, R.D., and Carmichael, W.W. 1989. Production and characterization of antibodies against microcystins. Appl. Environ. Microbiol. **55**: 1928–1933. PMID:2506810.

Codd, G.A. 1984. Toxins of freshwater cyanobacteria. Microbiol. Sci. **1**: 48–52. PMID:6444183.

Codd, G., and Bell, S.G. 1985. Eutrophication and toxic cyanobacteria in water. Sci. Technol. **21**: 1–13.

Codd, G.A., and Poon, G.K. 1988. Cyanobacterial toxins. *In* Biochemistry of the algae and cyanobacteria. Proceedings of the Phytochemistry Society of Europe. Vol. 28. *Edited by* L.J. Rogers and J.R. Gallon. Oxford University Press, Oxford. pp. 283–296.

Dahms, H.U., Ying, X., and Pfeiffer, C. 2006. Antifouling potential of cyanobacteria: a minireview. Biofouling, **22**: 317–327. doi:10.1080/08927010600967261. PMID:17110355.

Dias, E., Pereira, P., and Franca, S. 2002. Production of paralytic shellfish toxins by *Aphanizomenon* sp. LMECYA 31 (cyanobacteria). J. Phycol. **38**: 305–308.

Doan, N.T., Rickards, R.W., Rothschild, J.M., and Smith, G.D. 2000. Allelopathic actions of the alkaloid 12-epi-haplindole E isonitrile and calothrixin A from cyanobacteria of the genera *Fischerella* and *Calothrix.* J. Appl. Phycol. **12**: 409–416. doi:10.1023/A:1008170007044.

Dow, C.S., and Swoboda, U. 2000. Cyanotoxins. *In* The ecology of cyanobacteria. *Edited by* B.A. Whitton and M. Potts. Kluwer Academic Publishers, Netherlands. pp. 613–632.

Dow, C.S., Swoboda, U.K., and Firth, P. 1994. Release and degradation of microcystin during a *Microcystis aeruginosa* bloom in a fresh water reservoir. *In* Detection methods for cyanobacterial toxins. Proceedings of the First International Symposium on Detection Methods for Cyanobacterial (Blue Green Algal) Toxins. 27–29 September 1993. *Edited by* G.A. Codd, T.M. Jeffries, C.W. Keevil, and E. Potter. The Royal Society of Chemistry, Cambridge. pp. 158–160.

Etchegaray, A., Rabello, E., Dieckmann, R., Moon, D.H., Fiore, M.F., Dohren, H.V., Tsai, S., and Neilan, B.A. 2004. Algicide production by the filamentous cyanobacterium *Fischerella* CENA 19. J. Appl. Phycol. **16**: 237–243. doi:10.1023/B:JAPH.0000048509.77816.5e.

Falch, B.S., Konig, M.K., Wrigh, D., and Sticher, O. 1993. Ambigol A and B: new biologically active polychlorinated aromatic compounds from the terrestrial blue green alga *Fischerella ambigua.* J. Org. Chem. **58**: 6570–6575. doi:10.1021/jo00076a013.

Falconer, I. 1996. Potential impact on human health of toxic cyanobacteria. Phycologia, **35**: 74–79.

Fergusson, K.M., and Saint, C.P. 2000. Molecular phylogeny of *Anabaena circinalis* and its identification in environmental samples by PCR. Appl. Environ. Microbiol. **66**: 4145–4148. doi:10.1128/AEM.66.9.4145-4148.2000. PMID:10966445.

Frankmolle, W.P., Larsen, L.K., Caplan, F.R., Patterson, G.M.L., Knubel, G., and Moore, R.E. 1992. Antifungal cyclic peptides from the terrestrial blue green alga *Anabaena laxa.* J. Antibiot. (Tokyo), **45**: 1458–1466. PMID:1429232.

Fujiki, H., Sugimura, T., and Moore, R.E. 1983. New classes of environmental tumor promoters: indole alkaloids and polyacetates. Environ. Health Perspect. **50**: 85–90. doi:10.2307/3429537. PMID:6409606.

Gentile, J.H. 1971. Blue green algal toxins. *In* Microbial toxins. Vol. 7. *Edited by* S. Kadis, A. Ciegler, and S. Ajl. Academic Press, New York. pp. 27–66.

Gentile, J.H., and Maloney, T.E. 1969. Toxicity and environmental requirements of a strain of *Aphanizomenon flos-aquae* (L.) Ralfs. Can. J. Microbiol. **15**: 165–173. doi:10.1139/m69-028. PMID:5764275.

Gerwick, W.H., Roberts, M.A., Proteau, P.J., and Chen, J.L. 1994. Screening cultured marine microalgae for anticancer type activity. J. Appl. Phycol. **6**: 143–149. doi:10.1007/BF02186068.

Ghasemi, Y., Tabatabai, Y., Shokravi, S., Soltani, N., and Zarrini, G. 2003. Antifungal and antibacterial activity of paddy-fields cyanobacteria from the north of Iran. J. Sci. IR Iran, **14**: 1016–1104.

Gleason, F.K., and Baxa, C.A. 1986. Activity of the natural algicide, cyanobacterin, on eukaryotic microorganisms. FEMS Microbiol. Lett. **68**: 77–81.

Gleason, F.K., and Case, D.E. 1986. Activity of the natural algicide, cyanobacterin, on angiosperms. Plant Physiol. **80**: 834–838. PMID:16664727.

Gleason, F.K., and Paulson, J.L. 1984. Site of action of natural algicida, cyanobacterin in blue green alga, *Synechococcus* sp. Arch. Microbiol. **138**: 273–277. doi:10.1007/BF00402134.

Gorham, P.R. 1964. Toxic algae. *In* Algae and man. *Edited by* D.F. Jackson. Plenum Publishing Corp., New York. pp. 307–336.

Gross, E. 1999. Allelopathy in benthic and littoral areas: case studies on allelochemicals of benthic cyanobacteria and submerged macrophytes. *In* Principles and practices in plant ecology: allelochemical interactions. *Edited by* Inderjit, K.M.M. Dakshini, and C.L. Foy. CRC Press, Boca Raton, Florida, USA. pp. 179–199.

Gross, E.M. 2003. Allelopathy of aquatic autotrophs. Crit. Rev. Plant Sci. **22**: 313–339.

Gross, E.M., Wolk, C.P., and Jüttner, F. 1991. Fischerellin, a new allelochemical from the freshwater cyanobacterium *Fischerella muscicola*. J. Phycol. **27**: 686–692.

Hagmann, L., and Jüttner, F. 1996. Fischerellin A, a novel photosystem-II inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity. Tetrahedron Lett. **37**: 6539–6542. doi:10.1016/0040-4039(96)01445-1.

Harada, K. 1996. Chemistry and detection of microcystins. *In* Toxic *Microcystis. Edited* by M.F. Watanabe, H. Harada, W.W. Carmichael, and H. Fujiki. CRC Press, London, UK. pp. 79–102.

Harada, K.I., Ohtani, I., Iwamota, K., Suzuki, M., Watanabe, M.F., and Terao, K. 1994. Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method. Toxicon, **32**: 73–84. doi:10.1016/0041-0101(94)90023-X. PMID:9237339.

Harrigan, G.G., Luesch, H., Yoshida, W.Y., Moore, R.E., Nagle, D.G., Paul, V.J., et al. 1998. Symplostatin 1: a dolastatin 10 analog from the marine cyanobacterium, *Symploca hydnoides.* J. Nat. Prod. **61**: 1075–1077. doi:10.1021/np980321c. PMID:9748368.

Hirata, K., Yashitomi, S., Dwi, S., Iwabe, O., Mahakhant, A., Polchai, J., and Mivamota, K. 2003. Bioactivities of nostocine A produced by a freshwater cyanobacterium *Nostoc spongiaeforme* TISTR 8169. J. Biosci. Bioeng. **95**: 512–517. PMID:16233448.

Hrouzek, P., Kopecky, J., Salat, J., Marsalek, B., and Lukesova, L. 2005. Cytotoxic effect of soil cyanobacterial extracts to mammal cell lines YAC-1 and WEHI. Czech. Phycol. **5**: 79–90.

Hunter, P.R. 1995. Cyanobacterial toxins and their potential risk to drinking water supplies. Microbiol. Eur. **3**: 8–10.

Jaiswal, P., Prasanna, R., and Singh, P.K. 2005. Isolation and screening of rice field cyanobacteria exhibiting algicidal activity. Asian J. Microbiol. Biotechnol. Environ. Sci. **7**: 367–373.

Jaiswal, P., Prasanna, R., and Singh, P.K. 2007. Factors influencing algicide production by *Microcystis* sp., and its effect on selected cyanobacteria. *In* Advances in applied phycology. *Edited by* R.K. Gupta and V.D. Pandey. Daya Publishing House, New Delhi. pp. 75–84.

Jung, J.H., Moore, R.E., and Patterson, G.M.L. 1991. Scytophycins from a blue green alga belonging to the Nostocaceae. Phytochemistry, **30**: 3615–3616. doi:10.1016/0031-9422(91)80077-E.

Jüttner, F., Todororova, A.K., Walch, N., and Von Philipsborn, W. 2001. Nostocyclamide M: a cyanobacterial cyclic peptide with allelopathic activity from *Nostoc* 31. Phytochemistry, **57**: 613–619. doi:10.1016/S0031-9422(00)00470-2. PMID:11394868.

Kaebernick, M., Neilan, B.A., Berner, T., and Dittmann, T. 2000. Light and transcriptional response of the microcystin biosynthesis gene clustur. Appl. Environ. Microbiol. **66**: 3387–3392. doi:10.1128/AEM.66.8.3387-3392.2000. PMID:10919796.

Kaebernick, M., Dittmann, E., Berner, T., and Neilan, B.A. 2002. Multiple alternate transcripts direct the biosynthesis of microcystin, a cyanobacterial nonribosomal peptide. Appl. Environ. Microbiol. **68**: 449–455. doi:10.1128/AEM.68.2.449-455.2002. PMID:11823177.

Kaya, K., Mahakhant, A., Keovara, L., Sano, T., Kubo, T., and Takaji, H. 2002. Spiroidesin, a novel lipopeptide from the cyanobacterium *Anabaena spiroides* that inhibits cell growth of the cyanobacterium *Microcystis aeruginosa.* J. Nat. Prod. **65**: 920–921. doi:10.1021/np010660x. PMID:12088439.

Kearns, K.D., and Hunter, M.D. 2001. Toxin producing *Anabaena flos aquae* induces settling of *Chlamydomonas reinhardtii*, a competing motile alga. Microb. Ecol. **42**: 80–86. PMID:12035083.

Keating, K.I. 1977. Allelopathic influence on blue-green bloom sequence in a eutrophic lake. Science, **196**: 885–887. doi:10.1126/science.196.4292.885. PMID:17821808.

Keletis, G., and Sykora, J.L. 1982. Production and properties of cyanobacterial endotoxins. Appl. Environ. Microbiol. **43**: 104–109. PMID:6798930.

Kiviratna, J., and Abdel-Hameed, A. 1994. Toxicity of blue green alga *Oscillatoria agardhii* to the mosquito *Ades aegypti* and Shrimp *Artemia salina.* World J. Microbiol. Biotechnol. **10**: 517–520.

Kiviranta, J., Sivonen, K., and Niemela, S.I. 1991. Detection of toxicity of cyanobacteria by *Artemia salina* bioassay. Environ. Toxicol. Water Qual. **6**: 423–436. doi:10.1002/tox.2530060407.

Kondo, F., Ikai, Y., Oka, H., Ishikawa, N., Watanabe, M.F., Watanabe, M., Harada, K.-I., and Suzuki, M. 1992. Separation and identification of microcystins in cyanobacteria by frit-fast

atom bombardment liquid chromatography/mass spectrometry. Toxicon, **30**: 227–237. doi:10.1016/0041-0101(92)90865-3. PMID:1529459.

Kondo, F., Ikai, Y., Oka, H., Matsumoto, H., Yamada, S., Ishikawa, N., et al. 1995. Reliable and sensitive method for determination of microcystins in complicated matrices by fritfast atom bombardment liquid chromatography/mass spectrometry. Nat. Toxins, **3**: 41–49. doi:10.1002/nt.2620030109. PMID:7749582.

Kondo, F., Matsumoto, H., Yamada, S., Ishikawa, N., Ito, N., Nagata, S., et al. 1996. Detection and identification of metabolites of microcystins formed in vivo in mouse and rat livers. Chem. Res. Toxicol. **9**: 1355–1359. doi:10.1021/tx960085a. PMID:8951240.

Konz, D., and Marahiel, M.A. 1999. How do peptide synthetases generate structural diversity? Chem. Biol. **6**: R39–R48. doi:10.1016/S1074-5521(99)80002-7. PMID:10021423.

Kuiper-Googman, T., Falconer, I., and Fitzgerald, J. 1999. Human health aspects. *In* Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. *Edited by* I. Chorus and J. Bartram. Published on behalf of the World Health Organization by E. Spon and F.N. Spon, London, UK. pp. 113–154.

Kulik, M.M. 1995. The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur. J. Plant Pathol. **101**: 585–599. doi:10.1007/BF01874863.

Kurmayer, R., Christiansen, G., Fastner, J., and Borner, T. 2004. Abundance of active and inactive microcystin genotype in populations of toxic cyanobacterium *Planktothrix*. spp. Environ. Microbiol. **6**: 831–841. doi:10.1111/j.1462-2920.2004.00626.x. PMID:15250885.

Kusumi, K. 1996. Toxicology of microcystins. *In* Toxic *Microcystis. Edited by* M.F. Watanabe, H. Harada, W.W. Carmichael, and H. Fujiki. CRC Press, London, UK. pp. 149–174.

Kwan, J.C., Rocca, J.R., Abboud, K.A., Paul, V.J., and Luesch, H. 2008. Total structure determination of grassypeptolide, a new marine cyanobacterial cytotoxins. Org. Lett. **10**: 789–792. doi:10.1021/ol702946d. PMID:18220404.

Lambert, T.W., Boland, M.P., Holmes, C.F.B., and Hrudey, S.E. 1994. Quantification of microcystin hepatotoxins in water at environmentally relevant concentrations with the protein phosphatase bioassay. Environ. Sci. Technol. **28**: 753–755. doi:10.1021/es00053a032.

Leflaive, J., and Ten-Hage, L. 2007. Algal and cyanobacterial secondary metabolites in freshwater: a comparison of allelopathic compounds and toxins. Freshw. Biol. **52**: 199–214. doi:10.1111/j.1365-2427.2006.01689.x.

Legrand, C., Rengefors, K., Fistarol, G.O., and Graneli, E. 2003. Allelopathy in phytoplankton: biochemical, ecological and evolutionary aspects. Phycologia, **42**: 406–419.

MacKintosh, C., Beattie, K.A., Klump, S., Cohen, P., and Codd, G.A. 1990. Cyanobacterial microcystein-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett. **264**: 187–192. doi:10.1016/0014-5793(90)80245-E. PMID:2162782.

Matsunaga, S., Moore, R.E., Niemczura, W.P., and Carmichael, W.W. 1989. Anatoxin-a(s), a potent anticholinesterase from *Anabaena flos-aquae*. J. Am. Chem. Soc. **111**: 8021–8023. doi:10.1021/ja00202a057.

McElhiney, J., Lawton, L.A., Edwards, C., and Gallacher, S. 1998. Development of bioassay employing the desert locust (*Schistocera gregaria*) for the detection of saxitoxin and related compounds in cyanobacteria and shellfish. Toxicon, **36**: 417–420. doi:10.1016/S0041-0101(97)00077-9. PMID:9620590.

Mikalsen, B., Boisen, G., Skulberg, O.M., Fastner, J., Davis, W., Gabrielsen, T.M., Rudi, K., and Jacobsen, K.S. 2003. Natural variation in microcystin synthetase operon *mcyABC* and impact on microcystin production in *Microcystis* strains. J. Bacteriol. **185**: 2774–2785. doi:10.1128/JB.185.9.2774-2785.2003. PMID:12700256.

Moffitt, M.C., and Neilan, B.A. 2004. Characterization of nodularin synthatase gene clustur and proposed theory of the evolution of cyanobacterial hepatotoxin. Appl. Environ. Microbiol. **70**: 6353–6362. doi:10.1128/AEM.70.11.6353-6362.2004. PMID:15528492.

Moore, R.E. 1996. Cyclic peptides and depsipeptides from cyanobacteria: a review. J. Ind. Microbiol. **16**: 134–143. doi:10.1007/BF01570074. PMID:8730577.

Moore, R.E., Cheuk, C., Yang, X.G., and Pattreson, G.M.L. 1984. Hapalindoles: new alkaloids from blue green alga *Hapalosiphon fontinalis.* J. Am. Chem. Soc. **106**: 6456–6457. doi:10.1021/ja00333a079.

Moore, R.E., Patterson, G.M.L., Entzeroth, M., Morimoto, H., Suganuma, M., Hakii, H., Fujiki, H., and Sugimura, T. 1986. Binding studies of [H-3] lyngbyatoxin A and [H-3] debromoplysiatoxin to the phorbol ester receptor in a mouse epidermal particulate fraction. Carcinogenesis, **7**: 641–644. doi:10.1093/carcin/7.4.641. PMID:3698197.

Moore, R.E., Furusawa, E., Norton, T.R., Patterson, G.M.L., and Mynderse, J.S. 1989. Scytophycins. U.S. Patent No. 4,863,955.

Mundt, S., Kreitlow, S., Nowotny, A., and Effmert, U. 2001. Biochemical and pharmacological investigations of selected cyanobacteria. Int. J. Hyg. Environ. Health, **203**: 327–334. doi:10.1078/1438-4639-00045. PMID:11434213.

Nagata, S., Soutome, H., Tsutsumi, T., Hasegawa, A., Sekizima, M., Sugamata, M., and Uena, Y. 1995. Novel monoclonal antibodies against microcystin and their protective activity for hepatotoxicity. Nat. Toxins, **3**: 78–86. doi:10.1002/nt.2620030204. PMID:7613739.

Nagle, D.G., and Wedge, D.E. 2002. Antifungal properties of cyanobacteria and algae: ecological and agricultural implications. *In* Chemical ecology of plants: allelopathy in aquatic and terrestrial ecosystems, Birkhauser. *Edited by* Inderjit and A.U. Malik. Verlag, Switzerland. pp. 7–32.

Namikoshi, M., and Rinehart, K.L. 1996. Bioactive compounds produced by cyanobacteria. J. Ind. Microbiol. Biotechnol. **17**: 373–384.

Neilan, B.A., Jacobs, D., and Goodman, A.E. 1995. Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. Appl. Environ. Microbiol. **61**: 3875–3883.

Neuhof, T., Schmieder, P., Preussel, K., Dieckmann, R., Pham, H., Bartl, F., and Von Dohren, H. 2005. Hassallidin A, a glycosylated lipopeptide with antifungal activity from the cyanobacterium *Hassallia* sp. J. Nat. Prod. **68**: 695–700. doi:10.1021/np049671r. PMID:15921412.

Nianjun, X., Fan, X., Yan, X., and Tseng, C.K. 2004. Screening marine algae from China for their antitumor activities. J. Appl. Phycol. **16**: 451–456.

Nishizawa, T., Asayama, M., Fujii, K., Harada, K., and Shirai, M. 1999. Genetic analysis of the peptide synthetase genes for a cyclic heptapeptide microcystin in *Microcystis* spp. J. Biochem. (Tokyo), **126**: 520–529.

Nishizuka, Y. 1984. The role of protein kinase C in cell surface transduction and tumor promotion. Nature, **308**: 693–698. doi:10.1038/308693a0. PMID:6232463.

Oh, H.M., Lee, S.J., Jang, M.H., and Yoon, B.D. 2000. Microcystin production by *Microcystis aeruginosa* in a phosphorus limited

chemostat. Appl. Environ. Microbiol. **66**: 176–179. PMID: 10618220.

Ohtani, I., Moore, R.E., and Runnegar, M.T.C. 1992. Cylindrospermopsin: a potent hepatotoxin from the blue green alga *Cylindrospermopsis raciborskii.* J. Am. Chem. Soc. **114**: 7942–7944.

Ouellette, A.J., Handy, S.M., and Wilhelm, S.W. 2006. Toxic *Microcystis* is widespread in Lake Erie: PCR detection of toxin genes and molecular characterization of associated cyanobacterial communities. Microb. Ecol. **178**: 421–427.

Papke, U., Gross, E.M., and Francke, W. 1997. Isolation, identification and determination of the absolute configuration of Fischerellin B. A new algicide from the freshwater cyanobacterium *Fischerella miscicola* (Thuret.). Tetrahedron Lett. **38**: 379–382. doi:10.1016/S0040-4039(96)02284-8.

Patterson, G.M.L., and Bolis, C.M. 1993. Regulation of scytophycin accumulation in cultures of *Scytonema ocellatum*. I. Physical factors. Appl. Microbiol. Biotechnol. **40**: 375–381.

Patterson, G.M.L., and Carmeli, S. 1992. Biological effects of tolytoxin (6-hydroxy-7-*O*-methyl-scytophycin b) a potent bioactive metabolite from cyanobacteria. Arch. Microbiol. **157**: 406–410. doi:10.1007/BF00249096. PMID:1510566.

Patterson, G.M.L., Larsen, L.K., and Moore, R.E. 1994. Bioactive natural products from blue green algae. J. Appl. Phycol. **6**: 151–157. doi:10.1007/BF02186069.

Patterson, G.M.L., Moore, R.E., Carmeli, S., Smith, C.D., and Kimura, L.H. 1995. Scytophycin compounds, composition and methods for their production and use. U.S. Patent No. 5,493,933.

Peary, J.A., and Gorham, P.R. 1966. Influence of light and temperature on growth and toxin production by *Anabaena flos-aquae.* J. Phycol. **2**: 3.

Pouria, S., deAndrade, A., Barbosa, J., Caavalcanti, R.L., Barreto, V.T.S., Ward, C.J., et al. 1998. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. Lancet, **352**: 21–26. doi:10.1016/S0140-6736(97)12285-1. PMID:9800741.

Prasanna, R., and Jaiswal, P. 2006. Algal toxins — boon or bane to mankind. Sci. Cult. **72**: 9–10.

Pushparaj, B., Pelosi, E., and Caroppo, S. 2000. Effect of *Nodularia harveyana* biomass on the incidence of root-knot nematode *(Meloidogyne incognita*) in tomato. J. Appl. Phycol. **12**: 489–492. doi:10.1023/A:1008158711743.

Radhakrishnan, B. 2006. Potential of cyanotoxin as an antifungal agent. M.Sc. thesis, Division of Microbiology, Indian Agricultural Research Institute, New Delhi.

Rapala, J., and Sivonen, K. 1998. Assessment of environmental condition that favour hepatotoxic and neurotoxic *Anabaena* sp. strain in cultured under light limitation at different temperature. Microb. Ecol. **36**: 181–192. doi:10.1007/s002489900105. PMID:9688780.

Rapala, J., Sivonen, K., Luukkainen, R., and Niemela, S.I. 1993. Anatoxin-a concentration in *Anabaena* and *Aphanizomenen* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena* strains — a laboratory study. J. Appl. Phycol. **5**: 581–591. doi:10.1007/BF02184637.

Rapala, J., Sivonen, K., Lyra, C., and Niemela, S.I. 1997. Variation of microcystin, cyanobacterial hepatotoxin in *Anabaena* spp. as a function of growth stimuli. Appl. Environ. Microbiol. **64**: 2206–2212.

Ray, S., and Bagchi, S.N. 2001. Nutrients and pH regulate algicide accumulation in cultures of cyanobacterium *Oscillatoria latevirens.* New Phytol. **149**: 455–460. doi:10.1046/j.1469-8137.2001.00061.x.

Repka, S., Meyerhofer, M., Von Brockel, K., and Sivonen, K. 2004. Associations of cyanobacterial toxin nodularin with environmental factors and zooplanktons in the Baltic sea. Microb. Ecol. **47**: 350–358. PMID:14681737.

Ressom, R., Soong, F.S., Filzgerald, J., Turezynowicz, L.E.I., Saadi, O., Roder, D., Maynard, T., and Falconer, I. 1994. Health effects of toxic cyanobacterial (Blue Green Algae). National Health and Medical Research Council (NHMRC), Australia, Canberra Australia. Australian Government Publishing Service.

Rinehart, K.L., Harada, K., Namikoshi, M., Chen, C., Harvis, C.A., Munro, M.H.G., et al. 1988. Nodularin, microcystin and the configuration of Adda. J. Am. Chem. Soc. **110**: 8557–8558. doi:10.1021/ja00233a049.

Rouhianen, L., Vakkilainen, T., Siemer, B.L., Buikema, W., Haselkorn, R., and Sivonen, K. 2004. Genes coding for hepatotoxic heptapeptide (microcystins) in the cyanobacterium (*Anabaena* strain 90). Appl. Environ. Microbiol. **70**: 686–692. PMID:14766543.

Sawyer, P.J., Gentile, J.H., and Sasner, J.J., Jr. 1968. Demonstration of a toxin from *Aphanizomenon flos-aquae* (L.) Ralfs. Can. J. Microbiol. **14**: 1199–1204. PMID:5724889.

Schlegel, I., Doan, N.T., de Chazal, N., and Smith, G.D. 1999. Antibiotic activity of new cyanobacterial isolates from Australia, Asia against green algae and cyanobacteria. J. Appl. Phycol. **10**: 471–479.

Singh, D.P., Tyagi, M.B., Thakur, J.K., and Kumar, A. 2005*a*. Antialgal activity of a hepatotoxin-producing cyanobacterium, *Microcystis aeruginosa.* World J. Microbiol. Biotechnol. **17**: 15–22.

Singh, S., Kate, B.N., and Banerjee, U.C. 2005*b*. Bioactive compounds from cyanobacteria and microalgae: an overview. Crit. Rev. Biotechnol. **25**: 73–95. doi:10.1080/07388550500248498. PMID:16294828.

Sivonen, K. 1990. Effects of light, temperature, nitrate, orthophosphate and bacteria on growth and hepatotoxin production by *Oscillatoria agardhii* strains. Appl. Environ. Microbiol. **56**: 2658–2666. PMID:2125814.

Sivonen, K. 1996. Cyanobacterial toxins and toxins production. Phycologia, **35**: 12–29.

Sivonen, K., and Jones, G. 1999. Cyanobacterial toxins. *In* Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. *Edited by* I. Chorus and J. Bartram. Published on behalf of the World Health Organization by E. Spon and F.N. Spon, London, UK. pp. 41–111.

Skovgaard, A., Legrand, C., Hansen, P.J., and Granéli, E. 2003. Effects of nutrient limitation on food uptake in the toxic haptophyte *Prymnesium parvum*. Aquat. Microb. Ecol. **31**: 259–265. doi:10.3354/ame031259.

Smith, G.D., and Doan, N.T. 1999. Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. J. Appl. Phycol. **11**: 337–344. doi:10.1023/A:1008115818348.

Smith, C.D., Zhang, X., Mooberry, S.L., and Moore, R.E. 1994. Crytophycin: a new anitmicrotubule agent active against drug-resistant cells. Cancer Res. **54**: 3779–3784. PMID:7913408.

Srivastava, A., Juttner, F., and Strasser, R.J. 1998. Action of the allelochemical, fischerellin A on photosystem II. Biochim. Biophys. Acta, **1364**: 326–336. PMID:9630706.

Srivastava, R., Ray, S., and Bagchi, S.N. 2001. Action of an algicide from a cyanobacterium, *Oscillatoria latevirens*, on photosystem II. J. Exp. Biol. **39**: 1268–1273.

Suikkanen, S., Fistaral, G.O., and Graneli, E. 2004. Allelopathic effects of the Baltic cyanobacteria *Nodularia spumigena*, *Aphanizomenon flos aquae* and *Anabaena lemmermannit* on algal monoculture. J. Exp. Mar. Biol. Ecol. **308**: 85–101. doi:10.1016/j.jembe.2004.02.012.

Suikkanen, S., Fistarol, G.O., and Granéli, E. 2005. Effect of cyanobacterial allelochemicals on a natural plankton community. Mar. Ecol. Prog. Ser. **287**: 1–9. doi:10.3354/meps287001.

Sukenik, A., Eshkol, R., Livne, A., Hadas, O., Rom, M., Tchernov, D., Vardi, A., and Kaplan, A. 2002. Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp. (cyanobacteria): a novel allelopathic mechanism. Limnol. Oceanogr. **47**: 1656–1663.

Swoboda, U.K., and Dow, C.S. 1994. Expression of cyanotoxins in environmental biomass containing species of *Oscillatoria. In* Detection methods for cyanobacterial toxins. Proceedings of the First International Symposium on Detection Methods for Cyanobacterial (Blue Green Algal) Toxins. 27–29 September 1993. *Edited by* G.A. Codd, T.M. Jeffries, C.W. Keevil, and E. Potter. The Royal Society of Chemistry, Cambridge. pp. 164–167.

Tillett, D., Parker, D.L., and 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 RNA and phycocyanin operon (phycocyanin intergenic spacer) phylogenies. Appl. Environ. Microbiol. **67**: 2810–2818. doi:10.1128/AEM.67.6.2810-2818.2001. PMID:11375198.

Tonk, L., Visser, P.M., Christiansen, G., Dittmann, E., Snelder, E.F.M., Wiedner, C., Mur, L.R., and Huisman, J. 2005. The microcystin composition of the cyanobacterium *Planktothrix agardhi* changes toward a more toxic variant with increasing light intensity. Appl. Environ. Microbiol. **71**: 5777–5781.

Trimurtulu, G., Ohtani, I., Patterson, G.M.L., Moore, R.E., Corbett, T.H., Valeriote, F.A., and Demchik, L. 1994. Total structures of cryptophycins, potent antitumor desipeptides from blue green alga *Nostoc* sp. strain GSV 224. J. Am. Chem. Soc. **116**: 4729–4737. doi:10.1021/ja00090a020.

Turell, M.J., and Middlebrook, J.L. 1988. Mosquito inoculation: an alternative bioassay for toxins. Toxicon, **26**: 1089–1094. doi:10.1016/0041-0101(88)90208-5. PMID:2907687.

Turner, P.C., Gammie, A.J., Hollinrake, K., and Codd, G.A. 1990. Pneumonia associated with contact with cyanobacteria. BMJ, **300**: 1440–1441. PMID:2116198.

Utkilen, H., and Gjolme, N. 1992. Toxin production by *Microcystis aeruginosa* as a function of light in continuous cultures and its ecological significance. Appl. Environ. Microbiol. **58**: 1321–1325. PMID:16348697.

Vaitomaa, J., Rantala, A., Halinen, K., Rouhianen, L., Tallberg, P., Mokelke, L., and Sivonen, K. 2003. Quantitative real time PCR for determination of microcystin and *Anabaena* in lakes. Appl. Environ. Microbiol. **69**: 7289–7297. doi:10.1128/AEM.69.12.7289-7297.2003. PMID:14660378.

Van der Westhuizen, A.J., and Eloff, J.N. 1983. Effect of culture age and pH of culture medium on growth and toxicity of the blue-green alga *Microcystis aeruginosa.* Z. Pflanzenphysiol. **110**: 157–163.

Van der Westhuizen, A.J., and Eloff, J.N. 1985. Effect of temperature and light on toxicity and growth of the blue-green alga *Microcystis aeruginosa* (UV-006). Planta, **163**: 55–59.

Van der Westhuizen, A.J., Eloff, J.N., and Kruger, G.H.J. 1986. Effect of temperature and light (fluence rate) on the composition of the toxin of the cyanobacterium, *Microcystis aeruginosa* (UV-006). Arch. Hydrobiol. **108**: 145–154.

Vardi, A., Schatz, D., Beeri, K., Motro, U., Sukenik, A., Levine, A., and Kaplan, A. 2002. Dinoflagellate–cyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. Curr. Biol. **12**: 1767–1772. doi:10.1016/S0960-9822(02)01217-4. PMID:12401172.

Volk, R.B. 2005. Screening of microalgal culture media for the presence of algicidal compounds and isolation and identification of two bioactive metabolites, excreted by the cyanobacteria *Nostoc insulare* and *Nodularia harveyana.* J. Appl. Phycol. **17**: 339–347. doi:10.1007/s10811-005-7292-7.

Volk, R.B. 2006. Antialgal activity of several cyanobacterial exometabolites. J. Appl. Phycol. **18**: 145–151. doi:10.1007/s10811-006-9085-z.

Volk, R.B. 2007. Studies on culture age versus exometabolite production in batch cultures of the cyanobacterium *Nostoc imsulare.* J. Appl. Phycol. **19**: 491–495.

Volk, R.B., and Furkert, F.H. 2006. Anti-algal, antibacterial and antifungal activity of two metabolites produced by cyanobacteria during growth. Microbiol. Res. **161**: 180–186. doi:10.1016/j.micres.2005.08.005. PMID:16427523.

Volk, R.B., and Mundt, S. 2006. Cytotoxic and non-cytotoxic exometabolites of the cyanobacterium *Nostoc insulare.* J. Appl. Phycol. **19**: 55–62.

Von Elert, E., and Juttner, F. 1997. Phosphorus limitation and not light controls the extracellular release of allelopathic compounds by *Trichormus doliolum.* Phycologia, **42**: 1796–1802.

Ward, C.J., Beattie, K.A., Lee, E.Y.C., and Codd, G.A. 1997. Colorimetric protein phosphatase inhibition assay of laboratory strains and natural blooms of cyanobacteria: comparisons with high performance liquid chromatographic analysis. FEMS Microbiol. Lett. **153**: 465–473. doi:10.1111/j.1574-6968.1997.tb12611.x. PMID:9271876.

Watanabe, M.F., and Oishi, S. 1985. Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. Appl. Environ. Microbiol. **49**: 1342–1344. PMID:3923932.

Weckesser, J., Drews, G., and Mayer, H. 1979. Lipopolysaccharides of photosynthetic prokaryotes. Annu. Rev. Microbiol. **33**: 215–239. doi:10.1146/annurev.mi.33.100179.001243. PMID:115382.

Weise, G., Drews, G., Jann, B., and Jann, K. 1970. Identification and analysis of lipopolysaccharides in cell walls of the blue-green algae *Anacystis nidulans*. Arch. Microbiol. **71**: 89–98.

Welker, M., and Von Dohren, H. 2006. Cyanobacterial peptides — nature's own combinational biosynthesis. FEMS Microbiol. Rev. **30**: 530–563. doi:10.1111/j.1574-6976.2006.00022.x. PMID:16774586.

Wicks, J.R., and Thiel, P.G. 1990. Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir. Environ. Sci. Technol. **24**: 1413–1418. doi:10.1021/es00079a017.

Wiegand, C., and Pflugmacher, S. 2005. Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. Toxicol. Appl. Pharmacol. **203**: 201–218. doi:10.1016/j.taap.2004.11.002. PMID:15737675.

Wilson, K.M., Schembri, A., Baker, P.D., and Saint, C.P. 2000. Molecular characterization of the toxic cyanobacterium *Cylindrospermum raciborskii* and design of a species specific PCR. Appl. Environ. Microbiol. **66**: 332–338. PMID:10618244.

Zingone, A.O., and Enevoldsen, H. 2000. The diversity of harmful algal blooms: a challenge for science and management. Ocean Coast. Manag. **43**: 725–748. doi:10.1016/S0964-5691(00)00056-9.