

Cyanobacterial Toxin

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Abstract

Cyanobacteria which are known as blue-green algae can be found widespread around the world such as in lakes, ponds, rivers and brackish waters. Both toxin and non-toxin producing cyanobacterial species have been reported to make blooms. In the case of cyanobacterial toxins (cyanotoxins), there are two known groups as cytotoxins and biotoxins. The biotoxins comprise the alkaloids such as anatoxin-a, anatoxin-a(s), saxitoxins, cylindrospermopsin or lipopolysaccharides, and the cyclic peptides such as microcystins and nodularins. Cyanotoxins are shown to cause acute lethal, acute, chronic and sub-chronic poisoning in wild and domestic animals, and human. In studies both biological and chemical methods are used to determine cyanotoxins. The specific methods for toxin classification can be summarized as; bioassays, ELISA (enzyme linked immunosorbent assay), PPIA (Protein phosphatase inhibition assay), and MMPB (2-Methoxy-1- methyl-3-phenylbuturic acid). Additionally, the methods that are used to determine of toxins are can be classified as follow; NMR (Nuclear magnetic Resonance, MS (Mass Spectrometry), HPLC (high performance liquid chromatography) coupled with either photodiode array (PDA), HPLC-UV (High-performance liquid chromatography), TLC (Thin layer chromatography), MALDI-TOF-MS (matrix assisted laser/desorption ionization- time-of-flight-mass spectrometry), LC-MS (Liquid chromatography hyphenated with electrospray ionization triple quadrupole mass spectrometry).

INTRODUCTION

Cyanobacteria, which are known as blue-green algae, are prokaryotic microorganisms that live in both freshwater, such as; in lakes, ponds, rivers, and reservoir, and in marine systems (Ouellette and Wilhelm, 2003). While cyanobacteria are one of the very few special groups that can perform oxygenic photosynthesis and respiration simultaneously in the same compartment; many cyanobacterial species are known to be able to fix nitrogen (Carmichael, 2001; Vermaas, 2001). Most of the references describe cyanobacteria as Gram-negative and Gram-negative cell wall of cyanobacteria has been previously shown by the electron microscopy (Stewart et al., 2006).

Cyanobacteria can be seen in single-celled form or colonial form such as filaments, sheets or even hollow balls. The light responses exhibited by cyanobacteria apparently are the adaptation mechanisms for maintaining optimal light regimens to support photosynthesis, as well as to avoid from burial caused by sedimentation. In addition to light responses, there have been three reports related with cyanobacterial chemotaxis, or chemotaxis-related behaviours (Costa et al., 2006).

Cyanobacteria "Blooms", which can be seen as blue-green, milky blue, green, reddish, or dark brown blooms, and scum,

typically occur in freshwater (Richardson and Castenholz 1989; Rivasseau et al., 1998). Nutrient-rich bodies of water can support the rapid growth of cyanobacteria.

Cyanobacterial blooms and their effects have been reported to be widespread, frequent and typically seasonal. Both toxin and non-toxin producing species are known to cause cyanobacterial blooms (Carmichael, 2001; Christiansen et al., 2008). They also produce many secondary metabolites that either are toxic or show bioactivity on other organisms. One of the surveys has shown that an average of 59% of blooms contains toxins, and hepatotoxic blooms are more common than the neurotoxic ones (Rantala et al., 2006).

Cyanotoxins have two groups as; cytotoxins and biotoxins and they are both known to be responsible for acute lethal, chronic and sub-chronic poisoning of wild and domestic animals and human (Molica et al., 2005) The biotoxins include the alkaloids such as anatoxin-a, anatoxin-a(s), saxitoxins, cylindrospermopsin or lipopolysaccharides, and the cyclic peptides such as microcystins, nodularins (Carmichael, 2001; Hitzfeld et al., 2000).

MICROCYSTIN AND NODULARIN

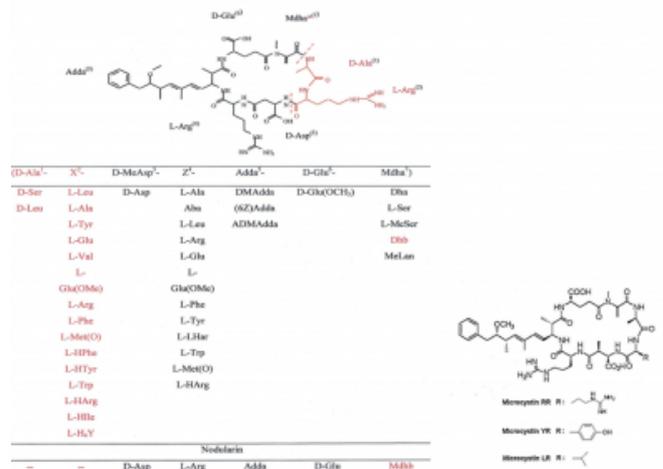
Microcystins are non-ribosomally synthesized via peptide synthetases, polyketide synthetases, and additional modifying enzyme system called microcystin synthetase

(Harada et al., 2004; Hotto et al., 2005). The enzyme complex consists of two operons transcribed bidirectionally from 10 internal promoter region. The first operon, mcyD–J, is responsible for the biosynthesis of the nonstandard amino acid Adda and for the incorporation of glutamic acid. The second operon contains mcyA–C gene which is responsible for the peptide synthetase genes that are encoded for the incorporation of the remaining amino acids (Hotto et al., 2005; Saker et al., 2005). The studies about variable hepatotoxicities reported that there are at least 76 different analogs in *Microcystis* and in other cyanobacteria (Anjos et al., 2006; Meriluoto and Codd, 2005). Microcystin-producing and non-microcystin-producing strains can be isolated from the same water (Furukawa et al., 2006). Cyanobacteria have been studied on the basis of their microscopic morphology. However, because different strains of the same species show identical morphologies and they could differ in toxigenicity; the identification and quantification of genus morphology is not eligible to determine the potential of microcystin production (Harada et al., 2004; Meriluoto and Codd, 2005).

Microcystins are the group of monocyclic heptapeptide (7 amino acids) hepatotoxins, containing a characteristic β -amino acid residue, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid (Adda), N-methyldehydroalanine (Mdha), D-alanine (Ala), β -linked D-erythro- β -methylaspartic acid (β -Me-Asp), and β -linked D-glutamic acid (Glu) which is most notably produced in both freshwater colonial cyanobacteria (*Microcystis aureginosa*, *Microcystis viridis*), and filamentous cyanobacteria (*Nostoc* spp., *Oscillatoria agardhii*- some of purified species are currently classified as *Planktothrix*- and *Anabaena foscuae*, *Hapalosiphon*) (Lemesa et al., 2008; Maagd et al., 1999; Tillett et al., 2000; Lawton and Edwards 2001). *Microcystis* structural variants detected per sample ranged from 2 (X) to 4 (Z) (Oberholster et al., 2004), and included microcystin-RR (contains the amino acids; Arginine (R) and Arginine (R)), -YR (tyrosine (Y) and Arginine (A)), -LR (Leucine (L) and Arginine (R)), LF (with phenylalanine group substituted in place of Arginine), LW (Leucine (L) and Tryptophan (W)) and LA (Leucine (L) and Alanine (A)) (Fig 1). The toxins are composed of five common amino acids and a pair of L- amino acids as variants (Watanabe et al., 1996; Allis et al, 2007; Mekebri et al., 2009).

Figure 1

Figure 1- Structure of a microcystin and nodularin (Christiansen ., 2003).



Microcystin and nodularin have variable components such as arginine or N-methyldehydroalanine (Mdha) (Pyo and Shin, 2001). Adda appears to have great importance in hepatotoxicity of microcystin and nodularins (Furukawa et al., 2006). While the L amino acid X has been most commonly found as leucine (L), arginine (R), or tyrosine(Y); alanine (A), homotyrosine (HTyr), phenylalanine (F), homophenylalanine (HPhe), methionine S-oxide [Met(O)], and tryptophan (W) variants of microcystins have also been detected. Correspondingly, while Z is arginine (R) or alanine (A); it might also be aminoisobutyric acid (Aba), homoarginine (HArg), or methionine S-oxide [Met(O)] (Fig 1) (Codd et al., 2001; Luukkainen et al., 1993).

Additionally a halotolerant organism, *Nodularia* was shown to have a strong potential for growth in estuaries; where it benefits from land-derive nutrients (Mazur and Plinski, 2003).

Cyanobacterial hepatotoxin nodularins (*Nodularia spumigena*) contain cyclic pentapeptide; β -linked D-glutamic acid, β -linked D-erythro- β -methylaspartic acid, L-arginine, 2-(methylamino)-2-dehydrobutyric acid (Mdhb), and Adda (Laamanen et al., 2001) (Fig 1). Currently, less than 10 analogues of nodularin have been isolated from natural blooms and laboratory cultures (Spoof and Meriluoto, 2002; Rapala et al., 2002).

Both nodularin and microcystins are relatively stable compounds that are not easily degraded by light, temperature or microwaves (Karjalainen, 2005). Intracellular cyanotoxins use freeze-drier or freeze-thawing for disruption of cell wall which is enhanced by ultrasonicator. While microcystins are

soluble in polar solvents (methanol and water); they cannot be dissolved in non-polar solvents. Extraction of microcystin has been achieved by using 100% methanol, methanol–water solutions, methanol–n-butanol–water, 5% acetic acid solutions –eliminate proteins and pigments in the material for chromatograph-, and supercritical fluid CO₂ modified with acetic acid. Similar extraction results were obtained with 25% methanol, n-butanol–methanol–water (5:20:75), n-butanol–methanol–acetic acid–water (5:20:1:74), methanol–acetic acid–water (25:1:74), and 5% acetic acid (Anjos et al., 2006; Aranda-Rodriguez et al., 2005).

The methods specific for toxin classification, can be summarized as; bioassays, ELISA (enzyme linked immunosorbent assay), PPIA (protein phosphatase inhibition assay), and MMPB (2-Methoxy-1- methyl-3-phenylbuturic acid). It is known that the toxicity of microcystis and nodularin are due to the inhibition of eukaryotic serine/threonine protein phosphatases (PPs), specifically PP1, PP2A and PP2B. Toxin specific methods, which are NMR (Nuclear magnetic Resonance, MS (Mass Spectrometry), HPLC (high performance liquid chromatography) coupled with either photodiode array (PDA) or high-performance capillary electrophoresis (HPCE), have been widely used for detection and quantitation of microcystins in water and in cyanobacterial cells (Hotto et al., 2007; Aguete et al., 2003). HPLC-UV (High-performance liquid chromatography), TLC (Thin layer chromatography), MALDI-TOF-MS (matrix assisted laser/desorption ionization- time-of-flight-mass spectrometry), Liquid chromatography hyphenated with electrospray ionization triple quadrupole mass spectrometry are also used as the detection methods for microcystins (Hotto et al., 2007; Allis et al., 2007; Karlsoon, 2005).

In 2003 a method was reviewed by the International Standards Organization (ISO/ CD 20179) and in this method an extraction procedure was recommended, that consist of three sequential extractions with 75% MeOH and sonication for 5 min. The most recent recommendation from 2004 included the sonication for 2 min on ice. According to the World Health Organization (WHO) guideline, the microcystin value in prohibited drinking water is 1 µg/L (ppb), which is based on microcystin-LR which is a specific microcystin toxin (Saker et al., 2005; Anjos et al., 2006).

Five pathways of microcystin detoxification can be considered for contributing to the natural routes as follow; 1. Dilution, 2. Absorption, 3. Thermal decomposition aided by temperature and pH, 4. Photolysis, and 5. Biological

degradation (Watanabe et al., 1996; Tsuji et al., 2001). In the case of biological degradation, Meriluoto et al. (2005) reported that the probiotics specific strains of lactobacilli and bifidobacteria can remove the cyanobacterial toxin microcystin-LR (Meriluoto et al., 2005).

CYLINDROSPERMOPSIN

Cylindrospermopsin [C₁₅H₂₁N₅O₇S] (MW= 415.43), a sulfate ester of a tricyclic guanidine substituted with a hydroxymethyluracil, is a cyanobacterial alkaloid toxin (Cylindrospermopsis raciborskii) and has increasing environmental import in drinking water reservoirs (Masten and Carson, 2000). The toxin has been isolated from cultures of Umezakia natans (in Japan), Raphidiopsis curvata (in China), Anabaena bergii (in Australia), Aphanizomenon ovalisporum (in Australia and Israel), Aphanizomenon flos-aquae (in Germany), Anabaena lapponica (in Finland), and Lyngbya wollei (in Australia). Cylindrospermopsin is also known as: 2,4(1H,3H)-Pyrimidinedione, 6-[(R)-hydroxy[2aS,3R,4S,5aS,7R) -2,2a,3,4,5,5a,6,7-octahydro-3-methyl-4-(sulfooxy) -1H-1,8,8b-triazaacenaphthylen-7-yl]methyl]; 1H-1,8,8b-Triazaacenaphthylene, 2,4(1H,3H)-pyrimidinedione deriv.; 2,4(1H,3H)-Pyrimidinedione, 6-[hydroxy[2,2a,3,4,5,5a,6,7-octahydro-3-methyl-4-(sulfooxy)-1H-1,8,8b-triazaacenaphthylen-7-yl]methyl]-2a,3,4,5a,7[(R*)]-(-); (-)-Cylindrospermopsine; Cylindrospermopsine (Masten and Carson, 2000).

As a toxin, cylindrospermopsin mainly targets liver but it has also effect on other organs. While cylindrospermopsin is a hepatocytes inhibitor of protein synthesis that causes widespread necrotic injury in human such as in liver, kidneys, lungs, spleen and intestine; it is also a hepatocytes inhibitor of glutathione (GSH) that leads to cell death. Glutathione (GSH) is an important non-protein thiol in the cell which has protective feature against oxidative damage and has important role in the detoxification. The mechanism of the decrease in GSH caused by cylindrospermopsin was assigned to the inhibition of GSH synthesis rather than to altered consumption (Reisner et al., 2004). It has genotoxic character because it can also cause chromosome loss and DNA strand breaks.

Cylindrospermopsin is a glassy solid and highly water-soluble toxin. The stability of cylindrospermopsin has been studied in different extreme conditions and cylindrospermopsin has been found to be relatively stable at very high temperatures (100 C for 15 minutes) and pH (pH

of 4, 7, and 10 for 8 weeks). Cylindrospermopsin is not commercially produced and is not used as a compound (Masten and Carson, 2000).

The analytical determination of cylindrospermopsin involves purification of cylindrospermopsin from purified extracts of cyanobacterial isolates with HPLC or over silica gel and characterization with mass spectrometry (MS) or NMR. In previous studies cylindrospermopsin was measured by HPLC using PDA detection and the results were confirmed by HPLC–MS (Runnegar et al., 2002). Sensitive and rapid analysis methods for water samples are currently investigated by using ELISAs, polymerase chain reaction (PCR) (Masten and Carson, 2000). Cylindrospermopsin was previously identified by reversed phase high-performance liquid chromatography-mass spectrometry (RP-HPLC or RPC) and tandem mass spectrometry (LC-MS/MS) with the use of an atmospheric-pressure ionization source and an ion spray interface (Stirling and Quilliam, 2001).

ANATOXIN

Anatoxins [C₁₀H₁₅NO] (MW = 165) are the group of low molecular weight neurotoxic alkaloids described in Table 1. They were found in the freshwater cyanobacteria *Anabaena flos-aquae*, *Planktothrix*, *Oscillatoria* and *Aphanizomenon*, *Woronichinia* (Puschner et al., 2008) and anatoxin-a was reported in brackish water, e.g. *Anabaena* was detected to make the bloom art of *Nodularia spumigena* in the Baltic Sea. Three common anatoxins have been described as neurotoxins. While anatoxin-a ((2-acetyl-9-azabicyclo[4.2.1] non-2- ene)) and homoanatoxin-a ((2-(propan-1-oxo-1-yl)-9-azabicyclo[4.2.1] non- 2-ene)) mimic the effect of acetylcholine and represent the secondary amines; anatoxin-a(S) (MW = 252) is an anticholinesterase and it is a unique phosphate ester of a cyclic N-hydroxyguanine produced by *Anabaena flos-aquae* and *A. lemmermannii* (Puschner et al., 2008; Araoz et al., 2005). These nicotinic agonist neurotoxins were investigated using isocratic micro-liquid chromatography-tandem mass spectrometry (micro-LC-MS-MS) and the compounds were described as; anatoxin-a and homoanatoxin-a, and their degraded products which are dihydroanatoxin-a, epoxyanatoxin-a, dihydrohomoanatoxin-a, and epoxyhomoanatoxin-a that are synthesized from the parent toxins (Viaggiu et al., 2004).

Figure 2

Table 1- Worldwide source of Anatoxins

Toxin	Cyanobacteria	Country
Anatoxin-a	<i>Anabaena flos-aquae</i>	Canada, Finland
	<i>Anabaena planktonia</i>	Italy
	<i>Anabaena</i> sp.	Ireland, Japan#
	<i>Oscillatoria</i>	Scotland, Ireland
	<i>Aphanizomenon</i>	Germany, Finland
	<i>Cylindrospermum</i>	Finland
	<i>Microcystis</i>	Japan
	<i>Planktothrix rubescens</i>	Italy
Homoanatoxin-a	<i>Oscillatoria formosa</i>	Norway
Anatoxin-a(S)	<i>Anabaena flos-aquae</i>	Canada, Denmark
	<i>Anabaena lemmermannii</i>	Denmark

(*Viaggiu et al., 2004; #Kele et al., 2006).

Anatoxin-a(S) similarly has action through synthetic organophosphonate nerve agents, such as sarin, soman or VX, which inhibit cholinesterases by phosphorylating their active site. While toxin is an acetylcholinesterase inhibitor; anatoxin-a(s) is not a homotropane (Wonnacott and Gallagher, 2006). Table 2 shows the different LD₅₀ values for cyanobacterial toxins.

Figure 3

Table 2- 50% lethal dose value (LD) for cyanobacterial toxins (Codd ., 2005);

Toxin	LD ₅₀ (i.p. mouse, µg/kg body wt)	Dominant genera
Anatoxin-a	250	<i>Anabaena</i> , <i>Oscillatoria</i> , <i>Aphanizomenon</i> , <i>Cylindrospermum</i> , <i>Planktothrix</i>
Homoanatoxin-a	250	<i>Planktothrix</i>
Anatoxin-a(s)	40	<i>Anabaena</i>
Saxitoxins	10-30	<i>Aphanizomenon</i> , <i>Planktothrix</i> , <i>Anabaena</i> , <i>Cylindrospermopsis</i> , <i>Lynghya</i>
Microcystins	25 to ~1000	<i>Microcystis</i> , <i>Anabaena</i> , <i>Nostoc</i> , <i>Anabaenopsis</i> , <i>Planktothrix</i> , <i>Hapalosiphon</i>
Nodularins	30-50	<i>Nodularia</i>
Cylindrospermopsin	200-2100	<i>Cylindrospermopsis</i> , <i>Aphanizomenon</i> , <i>Raphidiopsis</i> , <i>Umezakia</i>

Extraction of anatoxin-a has been performed by using water adjusted to pH 5 with HCL, 0.05 M acetic acid, or methanol. Anatoxin-a has been determined by HPLC after derivatization with 7- fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F) and the results were confirmed by HPLC–MS of the free or NBD-derivatized toxin (Runnegar et al., 2002).

Saxitoxins [C₁₀H₁₇N₇O₄] (MW = 299) are the carbamate alkaloids that block mammalian sodium channels and about 21 structural variants of sanitoxins have been found in cyanobacteria such as the strains of *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lynghya*, *Planktothrix* (Meriluoto and Codd, 2005).

Saxitoxins are known as the products of three marine dinoflagellate genera (*Alexandrium*, *Gymnodinium*, and *Pyrodinium*) which make the ‘red tides’ that leads to

paralytic shellfish poisoning (PSP) in human (Kele et al., 2006). The symptoms of PSP can be attributed to Saxitoxin and its congeners binding to sodium channels, which consequently block the influx of Na⁺ ions. This binding, subsequently, prevents nerve cells from producing action potentials and that inhibits impulse conduction in excitable tissues, e.g. muscle, peripheral nerve and brain tissues. Saxitoxin can dissolve in water and in lower alcohols; however it is known to be insoluble in organic solvents. Saxitoxin is stable in solutions at neutral and acidic pHs, even at high temperatures; however alkaline exposure oxidises and inactivates the toxin (Reisner et al., 2004).

The paralytic shellfish toxins (PSTs) are classified into carbamoyl or carbamate toxins, which are saxitoxin, neosaxitoxin, and gonyautoxins 1–4; and their N-sulfocarbamoyl derivatives as gonyautoxins 5, and 6, and C1–4; and finally decarbamoyl toxins. Small modifications of those elements are known as the result of sulfatation at positions of R2 and R3. PSTs are measured by HPLC with fluorescent detection after either chemical or electrochemical post-column derivatization (Runnegar et al., 2002).

Lipopolysaccharides (LPS) endotoxins are determined in several species or strains of cyanobacteria, including *Anabaena variabilis*, *Phormidium africanum*, *P. laminosum*, *P. uncinatum*, *Agmenellum quadruplicatum*, and *Schizothrix calcicola* (lipopolisaccaride) (Stewart et al., 2006; Jemmett et al., 2008).

Cyanobacterial LPS is known to cause a range of pathological effects in human, such as gastro-intestinal illness, cutaneous signs and symptoms, allergy, respiratory disease, headache, and fever (Stewart et al., 2006). While lipopolysaccharides contain polysaccharide and lipid; some authors have suggested that LPS should refer to a purified molecule such as endotoxin which more appropriately describes macromolecular complexes of LPS which is protein, phospholipid and nucleic acids (Stewart et al., 2006). These reviews are especially valuable because they have controversy data against the study done on other photosynthetic prokaryotes, some of which have been shown to have LPS antagonist lipid A structures. Cyanobacterial LPS also contain relatively large quantities of oleic, palmitoleic, linoleic, and linolenic acids, which typically are absent in gram-negative LPS molecules. Additionally, unlike lipid A in gram-negative LPS, lipid A in cyanobacteria does not contain phosphorus (Jemmett et al., 2008).

In conclusion, natural water has been used as fresh water, for

example as drinking water, aquapark water, and bath water. This review emphasizes that fresh water is a habitat for toxic cyanobacteria which make blooms and produces different toxins. While legal regulations about chemical analysis, such as heavy metals and pesticides, monitor water quality; also cyanobacterial toxin analysis must be taken into consideration for human health around the world.

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References

- r-0. Aguet EC, Gago-Martínez A, Leão JM, Rodríguez-Vázquez JA, Menard C, Lawrence JF: HPLC and HPCE analysis of microcystins RR, LR and YR present in cyanobacteria and water by using immunoaffinity extraction. *Talanta*; 2003; 59: 697–705.
- r-1. Allis O, Duphard J, Hamilton B, Shuilleabhain AN, Lehane M, James JK, Furey A: Liquid Chromatography-Tandem Mass Spectrometry Application, for the determination of Extracellular Hepatotoxins in Irish Lake and Drinking Waters. *Anal. Chem.*; 2007; 79:3436-3447.
- r-2. Anjos FM, Bittencourt-Oliveira MC, Zajaca MP, Hillerd S, Christiand B, Erlerd K, Luckasd B, Pinto E: Detection of harmful cyanobacteria and their toxins by both PCR amplification and LC-MS during a bloom event. *Toxicon*; 2006; 48: 239–245.
- r-3. Aranda-Rodriguez R., Tillmanns A, Benoit FM, Pick FR, Harvie J, Solenaia L: Pressurized Liquid Extraction of Toxins from Cyanobacterial Cells. *Environmental Toxicology*; 2005; 20: 390 - 396.
- r-4. Araoz R, Nghiem HO, Rippka R, Palibroda N, De Marsac NT, Herdman M: Neurotoxins in axenic oscillatory cyanobacteria: coexistence of anatoxin-a and homoanatoxin-a determined by ligand-binding assay and GC/MS. *Microbiology*; 2005; 151: 1263–1273.
- r-5. Carmichael, WW: Health Effects of Toxin-Producing Cyanobacteria: “The CyanoHABs”. *Human and Ecological Risk Assessment*; 2001; 7: 1393-1407.
- r-6. Christiansen G, Fastner J, Erhard M, Börner T, Dittmann E: Microcystin Biosynthesis in Planktothrix: Genes, Evolution, and Manipulation. *Journal of Bacteriology*; 2003; 185, 22, 564–572.
- r-7. Christiansen G, Molitor C, Philmus B, Kurmayer R: Nontoxic Strains of Cyanobacteria Are the Result of Major Gene Deletion Events Induced by a Transposable Element. *Mol. Biol. Evol.*; 2008; 25: 1695-1704.
- r-8. Codd GA, Metcalf JS, Ward CJ, Beattie KA, Bell SG, Kaya K, Poon KG: Analysis of cyanobacterial toxins by Physicochemical and biochemical methods. *Journal of AOAC International*; 2001; 84: 1626-35.
- r-9. Codd GA, Morrison LF, Metcalf JS: Cyanobacterial toxins: risk management for health protection. *Toxicology and Applied Pharmacology*; 2005; 203: 264-272.
- r-10. Costa IAS, Azevedo SMFO, Senna PAC, Bernardo RR, Costa SM, Chellappa NT: Occurrence of Toxin-Producing Cyanobacteria Blooms In A Brazilian Semiarid Reservoir. *Braz. J. Biol.*; 2006; 66(1B): 211-9.
- r-11. Furukawa K, Noda N, Tsuneda S, Saito T, Itayama T, Inamari Y: Highly Sensitive Real-Time PCR Assay for Quantification of Toxic Cyanobacteria Based on Microcystin Synthetase A Gene. *J. of Bioscience and Bioengineering*;

- 2006; 102: 90-96.
- r-12. Harada K, Nakano T, Fujii K, Shirai M: Comprehensive analysis system using liquid chromatography-mass spectrometry for the biosynthetic study of peptides produced by cyanobacteria. *Journal of Chromatography A*; 2004; 1033: 107-113.
- r-13. Hitzfeld BC, C.S. Lampert, N. Spaeth, D. Mountfort, H. Kaspar, and D.R. Dietrich: Toxin production in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. *Toxicon*; 2000; 38: 1731-1748.
- r-14. Hotto A, Satchwell M, Boyer G: Seasonal Production and Molecular Characterization of Microcystins in Oneida Lake, New York, USA. *Environmental Toxicology*; 2005; 20: 243-248.
- r-15. Hotto AM, Satchwell MF, Boyer GL: Molecular Characterization of Potential Microcystin-Producing Cyanobacteria in Lake Ontario Embayments and Nearshore Waters. *Applied and Environmental Microbiology*; 2007; 73: 4570-4578.
- r-16. Jemmett K, Macagno A, Molteni M, Heckels JE, Rossetti C, Christodoulides M: A Cyanobacterial Lipopolysaccharide Antagonist Inhibits Cytokine Production Induced by *Neisseria meningitidis* in a Human Whole-Blood Model of Septicemia. *Infection and Immunity*; 2008; 76: 3156-3163.
- r-17. Karjalainen M: Fate and effects of *Nodularia spumigena* and its toxin, nodularin, in Baltic Sea planktonic food webs, Finnish Institute of Marine Research, Finland Helsinki 1-34p (2005).
- r-18. Karlsoon K: Liquid Chromatography-Mass Spectrometry in Distribution and Bioaccumulation Studies of Toxic Cyanopeptides. Department of Biochemistry and Pharmacy Abo Akademi University (2005).
- r-19. Kele P, Orbulescu J, Gawley RE, Leblanc RM: Spectroscopic detection of Saxitoxin: an alternative to mouse bioassay. *Chem. Commun*; 2006; 14: 1494-1496.
- r-20. Laamanen MJ, Gugger MF, Lehtimäki JM, Haukka K, Sivonen K: Diversity Of Toxic And Nontoxic *Nodularia* Isolates (Cyanobacteria) and Filaments From The Baltic Sea. *Applied and Environmental Microbiology*; 2001; 67: 4638-4647.
- r-21. Lawton L, Edwards AC: Purification of microcystins, Review. *Journal of Chromatography A*; 2001; 912: 191-209.
- r-22. Lemesa GAF, Kersanacha R, Pintob L da S, Dellagostinb OA, Yunesa JS, Matthiensen A: Biodegradation of microcystins by aquatic Burkholderia sp. from a South Brazilian coastal lagoon. *Ecotoxicology and Environmental Safety*; 2008; 69: 358-365.
- r-23. Luukkainen R, Sivonen K, Namikoshi M, Fardig M, Rinehart KL, Niemela SI: Isolation and Identification Of Eight Microcystins From Thirteen *Oscillatoria agardhii* Strains and Structure of A New Microcystin. *Applied and Environmental Microbiology*; 1993; 59: 2204-2209.
- r-24. Maagd PGJD, Hendriks AJ, Seinen W, Sijm DTHM: Ph-Dependent Hydrophobicity of The Cyanobacteria Toxin Microcystin-Lr. *Wat. Res.*; 1999; 33: 677-680.
- r-25. Masten S, Carson B: *Cylindrospermopsin*- Review of Toxicological Literature. Toxicological Summary For *Cylindrospermopsin*. CASRN 143545-90-8, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709; 2000.
- r-26. Mazur, H, Plinski M, *Nodularia spumigena* blooms and the occurrence of hepatotoxin in the Gulf of Gdansk. *Oceanologia*; 2003; 45, 305-316.
- r-27. Mekebria A, Blondina GJ, Crane DB: Method validation of microcystins in water and tissue by enhanced liquid chromatography tandem mass spectrometry. *J. of Chromatography A*; 2009; 1216: 3147-3155.
- r-28. Meriluoto J, Codd GA: Toxic (Cyanobacterial Monitoring and Cyanotoxin Analysis) Abo Akademi University Pres, Turku/Åbo Finland 149 (2005).
- r-29. Meriluoto J, Gueimonde M, Haskard CA, Spool L, Sjövall O, Salminen S: Removal of the cyanobacterial toxin microcystin-LR by human probiotics. *Toxicon*; 2005; 46: 111-114.
- r-30. Molica RJR, Oliveira EJA, Carvalho PVVC, Costa ANSF, Cunha MCC, Melo GL, Azevedo SMFO: Occurrence of saxitoxins and an anatoxin-a(s)-like anticholinesterase in a Brazilian drinking water supply. *Harmful Algae*; 2005; 4: 743-753.
- r-31. Oberholster PJ, Botha A-M, Grobbelaar JU, *Microcystis aeruginosa*: source of toxic microcystins in drinking water-Review. *African Journal of Biotechnology*; 2004; 3:159-168.
- r-32. Ouellette A J, Wilhelm SW: Toxic cyanobacteria: the evolving molecular toolbox. *Front Ecol Environ*; 2003; 1:359-366.
- r-33. Puschner B, Hoff B, Tor ER: Diagnosis of anatoxin-a poisoning in dogs from North America. *J. Vet. Diagn. Invest.*; 2008; 20, 89-92.
- r-34. Pyo D, Shin H, Supercritical Fluid Extraction of microcystin Yr from cyanobacteria. *Analytical Sciences*; 2001; 17: 175-178.
- r-35. Rapala J, Erkomaa K, Kukkonen J, Sivonen K, Lahti K: Detection of microcystins with protein phosphatase inhibition assay, high-performance liquid chromatography-UV detection and enzyme-linked immunosorbent. *Analytica Chimica Acta*; 2002; 466: 213-231.
- r-36. Rantala A, Rajaniemi-Wacklin P, Lyra C, Lepistö L, Rintala J, Mankiewicz-Boczek J, Sivonen K: Detection of Microcystin-Producing Cyanobacteria in Finnish Lakes with Genus-Specific Microcystin Synthetase Gene E (mcyE) PCR and Associations with Environmental Factors. *Applied and Environmental Microbiology*; 2006; 72: 6101-6110.
- r-37. Reisner M, Carmeli S, Werman M, Sukenik A: The Cyanobacterial Toxin *Cylindrospermopsin* Inhibits Pyrimidine Nucleotide Synthesis and Alters Cholesterol Distribution in Mice. *Toxicological Sciences*; 2004; 82: 620-627.
- r-38. Richardsont LL, Castenholz RW: Chemokinetic Motility Responses of The Cyanobacterium *Oscillatoria terebriformis*. *Applied and Environmental Microbiology*; 1989; 55: 261-263.
- r-39. Rivasseau C, Martins S, Hennion MC: Determination of some physicochemical parameters of microcystins (cyanobacterial toxins) and trace level analysis in environmental samples using liquid chromatography. *Journal of Chromatography A*; 1998; 799 (1-2): 155-69.
- r-40. Runnegar MT, Xie C, Snider BB, Wallace GA, Weinreb SM, Kuhlenkamp J: In Vitro Hepatotoxicity of the Cyanobacterial Alkaloid *Cylindrospermopsin* and Related Synthetic Analogues. *Toxicological Sciences*; 2002; 67: 81-87.
- r-41. Saker ML, Jungblut A-D, Neilan BA, Rawn DFK, Vasconcelos VM: Detection of microcystin synthetase genes in health food supplements containing the freshwater cyanobacterium *Aphanizomenon flos-aquae*. *Toxicon*; 2005; 46: 555-562.
- r-42. Soares RM, Yuan M, Servaites JC, Delgado A, Magalhaes VF, Hilborn ED, Carmichael WW, Azevedo SMFO: Sublethal Exposure form Microcystins to Renal Insufficiency Patients in Rio de Janeiro, Brazil. *Environ Toxicol*; 2006; 21: 95-103.
- r-43. Spool L, Meriluoto J: Rapid separation of microcystins and nodularin using a monolithic silica C18 column. *J. of Chromatography A*; 2002; 947:237-245.

- r-44. Stewart I, Schluter PJ, Shaw GR: Cyanobacterial lipopolysaccharides and human health – a review. *Environmental Health: A Global Access Science Source*; 2006; 5:1-23.
- r-45. Stirling D, Quilliam MA: First report of the cyanobacteria toxin cylindrospermopsin in New Zealand. *Toxicon*; 2001; 39: 1219-1222.
- r-46. Tillett D, Dittmann E, Erhard M, von Döhren H, Börner T, Neilan B A: Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7804: an integrated Peptide-polyketide synthetase system. *Chemistry & Biology*; 2000; 7: 753-64.
- r-47. Tsuji K, Masui H, Uemura H, Mori Y, Harada K: Analysis of microcystins in sediments using MMPB method. *Toxicon*; 2001; 39: 687-692.
- r-48. Vermaas, WFJ: Photosynthesis and Respiration in Cyanobacteria. *Encyclopedia Of Life Sciences*, John Wiley & Sons, Ltd. www.els.net 1-7, 2001.
- r-49. Viaggiu E, Melchiorre S, Volpi F, Di Corcia A, Mancini R, Garibaldi L, Crichigno G, Bruno M: Anatoxin-A toxin in the cyanobacterium *Planktothrix rubescens* from a fishing pond in northern Italy. *Environmental Toxicology*; 2004; 19: 191-197.
- r-50. Watanabe MF, Harada K, Carmichael WW, Fujiki H: *Toxic Microcystis*, CRC Pres, Boca Raton, New York, London Tokyo 272, 1996.
- r-51. Wonnacott S, Gallagher T: *The Chemistry and Pharmacology of Anatoxin-a and Related Homotropanes with respect to Nicotinic Acetylcholine Receptors*. *Mar. Drugs*; 2006; 4: 228-254.
- r-52.

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