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Review

Methods for determining microcystins (peptide hepatotoxins) and microcystin-producing cyanobacteria

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ABSTRACT

Episodes of cyanobacterial toxic blooms and fatalities to animals and humans due to cyanobacterial toxins (CBT) are known worldwide. The hepatotoxins and neurotoxins (cyanotoxins) produced by bloom-forming cyanobacteria have been the cause of human and animal health hazards and even death. Prevailing concentration of cell bound endotoxin, exotoxin and the toxin variants depend on developmental stages of the bloom and the cyanobacterial (CB) species involved. Toxic and non-toxic strains do not show any predictable morphological difference. The current instrumental, immunological and molecular methods applied for determining microcystins (peptide hepatotoxins) and microcystin-producing cyanobacteria are reviewed.

Contents

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1. Introduction

Cyanobacterial growth leading to blooms along with toxin formation and their fatalities to livestock, pets, wild animals, aquatic animals, birds and humans are known worldwide (Carmichael, 1994; Harada et al., 2004; Jayatissa et al., 2006; Agrawal et al., 2006; Gkelis et al., 2006; Sotero-Santos et al., 2006; Jungblut et al., 2006). Cyanobacteria produce a wide range of toxic compounds, including hepatotoxins (such as the microcystins) and neurotoxins (such as the saxitoxins). Microcystins (MCs) are the most commonly encountered cyanotoxins (Sivonen, 1996). *Microcystis*, *Anabaena*, *Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*, etc. are common microcystin producing cyanobacterial genera (Carmichael, 1992; Sivonen and Jones, 1999). The toxin microcystin is produced nonribosomally via a multifunctional enzyme complex, consisting of both peptide synthetase and polyketide synthase modules coded by the mcy gene cluster (Neilan et al., 1999; Tillett et al., 2000). Microcystins are a group of closely related cyclic heptapeptides sharing the common structure cyclo (D-Ala-L-X-D-MeAsp-L-Z-Adda-D-Glu-Mdha), in which MeAsp is D-erythro-β-methylaspartic acid, Mdha is *N*-methyldehydroalanine, Adda is 2S, 3S, 8S, 9S-3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4E, 6E-dienoic acid, and *X* and *Z* are variable L-amino acids [e.g. microcystin-LR (MC-LR) contains leucine (L) and arginine (R)] (Carmichael et al., 1988). So far, more than 60 derivatives of microcystins have been identified, varying largely by the degree of methylation, peptide sequence, and toxicity (Sivonen and Jones, 1999). Microcystins can be classified into four groups according to the amino acid structure at unit 7. Microcystins normally contain *N*-methyldehydroalanine Mdha or dehydroalanine (Dha) at unit 7, and command the great part of all microcystins. Other classes include microcystins containing *N*-methyldehydro-butyrine (Dhb^7) or D- and L-Alanine or *N*-MethylAlanine (D- and L-Ala^7, or *N*-$MeAla^7$) or L-Serine (L-Ser^7) at unit 7 (Kaya et al., 2001).

The toxicity of microcystins is mediated through inhibition of serine-threonine protein phosphatases 1 and 2A (MacKintosh et al., 1990), which can cause both acute and chronic effects in mammals. Intoxication with high levels of microcystins can lead to hepatocyte necrosis and hemorrhage, with severe cases resulting in death (Bhattacharya et al., 1997). Long-term exposure to low concentrations of microcystins has also been implicated in tumor promotion (Ito et al., 1997). Guideline value for drinking water was introduced by the World Health Organization (WHO) (1998), with a recommended limit of 1 μg of MC-LR equivalents per liter. There is, therefore, a need for sensitive and robust detection methods for determining the levels of microcystins in water.

Development of reliable methods suitable for monitoring toxic CB and microcystins in environmental samples has been the quest of researchers. Variety and complexity of widely encountered microcystins warrant continued development of the procedures for greater analytical precision. No single technique, among the available ones, is sufficiently powerful to give both a precise measurement of toxicity and an accurate profile of the microcystin variants present (Harada et al., 1999). Microcystins are routinely monitored using analytical techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS) (Lawton et al., 1994a, 1995). However, these can be slow, technically demanding and often require extensive sample processing prior to analysis. A promising alternative for microcystin detection is the use of immunological assays employing either polyclonal (Chu et al., 1989; Metcalf et al., 2000; Sheng et al., 2006; Young et al., 2006) or monoclonal (Nagata et al., 1995) antibodies. The enzyme-linked immunosorbent assay (ELISA) is quick to perform, inexpensive, requires minimum sample processing, and is capable of detecting microcystins within the levels set by WHO (Hawkins et al., 2006). In addition to their application in immunoassay formats, microcystin-specific antibodies are also useful tools for processing complex samples prior to toxin analysis. They have been used effectively in immunoaffinity chromatography to eliminate contaminants from biological samples (Kondo et al., 1996; Mhadhbi et al., 2006) and to remove co-eluting compounds when microcystins are concentrated from large water samples (Kondo et al., 2000; Lawrence and Menard, 2001), thus enabling identification of the toxins by HPLC.

Water resource management has been complicated by the inability to differentiate between toxic and nontoxic cyanobacterial blooms without isolation and testing for toxin production. Toxic and non-toxic strains show no predictable difference in appearance and different strains of the same species may not be toxic or may produce different types of toxins (Yoo et al., 1995; Spoof et al., 2003). The level of toxin produced depends on the stage of blooms, species and strains involved which limit the microscopic examination for assessing the toxic nature of a sample. However, with the application of immunological, molecular and instrumental techniques, researchers made it possible to detect, differentiate, quantify and monitor the toxic cyanobacteria (CB) and toxins. Cell bound toxins (endotoxins) are mainly released when cells are lysed or become old and leaky (Lam et al., 1995) although active release of toxin (exotoxins) may also occur from young growing cells (WHO, 1998). The chemicals added for control or eradication of cyanobacterial blooms contribute towards release of endotoxins (Oberholster et al., 2004). Investigation of both cell bound microcystins (endotoxins) and exotoxins is important for assessment of total microcystins in waterbodies.

This article is an attempt to critically review the methods (Figs. 1 and 2), their suitability and limitations in analysis of microcystins and microcystin producing CB.

2. Methods for analysis of microcystins

2.1. *Extraction of endotoxins*

Cyanobacterial biomass, either from field or laboratory grown culture, is concentrated by centrifugation at 4000*g* for 20 min (Ward et al., 1997), net filtration through 10–64 μm mesh size plankton net (Kotak et al., 1996; Fastner et al., 1998; Karlsson et al., 2005), and micro-filtration using GF/C (Spoof et al., 2003) or by vacuum filtration using GF/B filter paper (Ortea et al., 2004). While aqueous (aq) phase is used for exotoxin analysis,