Detection of Cyanobacterial Toxins and Oligopeptides from the Polemidia Dam in Cyprus

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Abstract
Cyanobacterial harmful algal blooms are becoming more spatially persistent worldwide. The formation and release, through cell-death and/or excretion, of bioactive metabolites (cyanotoxins) is comprising a reoccurring concern to environmental protection agencies. Cyanotoxins can negatively impact mammalian health in various ways by causing cytotoxicity, hepatotoxicity, and neurotoxicity. Besides conventional toxins some genera can produce an array of oligopeptides characterized as microginin, cyanopeptolins, and aeruginosins. In this work we studied the occurrence of both conventional cyanotoxins as well as oligopeptides in a eutrophic dam located in Cyprus (Polemidia Dam) in years 2014-2018. To identify those oligopeptides advanced analytical techniques have been employed; specifically, tandem mass spectrometry at different modes (for screening and for quantitative analysis). Our initial results indicate that the detection of conventional cyanotoxins was lower than the method detection limits and for their detection in the ng/L range the analysis was conducted in the MRM mode. An array of oligopeptides was detected such as microcin SF608 (*m/z* 609.34), anabaenopeptin F (*m/z* 851.49), and aeruginosin 602 (*m/z* 609.34). There were no seasonal variations for the years 2014 and 2015 sampling events, while some of the oligopeptides (*m/z* 726.6) detected have not been previously reported in the cited literature.
Keywords: cyanotoxins, oligopeptides, tandem mass spectrometry, cyanobacteria, toxicity

1. Introduction

Toxic genera of cyanobacteria excrete and/or release into the water (through lysis) a broad variety of bioactive metabolites. These metabolites can negatively impact the ecosystem and human health in various ways, making it an important environmental issue of concern (Faltermann S. et al., 2014). Besides conventional cyanotoxins (microcystin, nodularin, and cylindrospermopsin), some genera can produce an array of oligopeptides classified as aeruginosins, anabaenopeptins, microcystins, microginins and other. These oligopeptides found to have key metabolic activities as shown on Table 1. Their toxicity is still under investigation, though studies have indicated that oligopeptides may inhibit key metabolic enzyme, mainly protein phosphatases and proteases but at a lesser extent to the hepatotoxic microcystins. However, some cyanopeptolines were found to have more lethal effect on small invertebrates than microcystins (M. Welker et al, 2006).

Table 1. The four major oligopeptide classes identified in samples and their main bioactivities (M. Welker et al., 2004).

Oligopeptides	Structure	Activity
Anabaenopeptins	Cyclic	Protein phosphatase, carboxypeptidase-A
Microcystins	Cyclic with Adda[a]	Protein phosphatases 1 and 2A
Microginins	Linear	Proteases, angiotensin-converting enzyme
Aeruginosins	Linear	Proteases, tyrosinase

The main objective of the study was the identification and quantification of both conventional cyanotoxins and oligopeptides (microginin, cyanopeptolins and aeruginosins) in a eutrophic dam located in Cyprus (Polemidia Dam) during 2014-2018. To achieve the identification of the toxic metabolites, tandem mass spectrometry was utilized at different modes.

2. Materials and Methods

Samples were collected during 2014-2019 for further analysis. Both surface and water column (euphotic zone) samples were taken with the assistance of the Water Development Department of Cyprus. Samples of 200-250 mL were filtrated through glass-fiber filters 47 mm (GF/C – 1.2μm) and stored in the freezer at -20˚C. Extraction of toxins and oligopeptides from the filter occurred with MeOH solution (50 % - 75%).

For the detection and quantification the analysis was performed on a UPLC system with photodiode array equipped with Tandem Quadruple Time of Flight in series (Waters,Elstree,UK). Samples were separated on

Acquity UPLC C18 column, maintained at 40°C. Instrumental control, data acquisition and processing were achieved using MassLynx software (Version4.1). In addition, an AB SCIEX QTRAP® 5500 System was utilized for obtaining mass spectra (Skowrońska B. et al.,2019). Results were collected and analyzed with Analyst Software®. For microcystins characterization the program "Cyanotox" Version 1.5.3 was used.

3. Results and Discussion

Samples collected from Polemidia Dam between 2014-2018 were analysed for conventional cyanotoxins (MCs) and oligopeptides. Oligopeptides detected between 2014-2015 include microcin SF608 (*m/z* 609.34), anabaenopeptin F (*m/z* 851.49) and aeruginosin 602 (*m/z* 603.35). These oligopeptides are formed by four to six peptides in cyclic or linear configurations (Table 1). For years 2017-2018 analysis indicated that the total intracellular concentration of MCs expressed as MC-LR equivalents did not exceed 150 ng/L. The MCs detected in water were: MC-LR, MC-RR, and MC-LF. Oligopeptides were also detected in samples taken during the same time period, with peptide sequences that correspond to aeruginosins, anabaenopeptins and microcystins (Table 2). Their sequence was determined through MS/MS spectra extraction from Analyst Software ®. An example of oligopeptide sequence determination is shown in Figure 1. The oligopeptide has been categorized as aeuroginosin based on its characteristic structure of **Cl-Pla-Leu-Choi(-Xyl)-Agm** since Pla and Choi groups are both characteristic peptides for this oligopeptide class. The peak at 726.6 corresponds to the mass-to-charge ratio of the total molecule. Other two peaks are present at 122, 140 which indicate the Choi group, while a peak at 114 indicate the presence of Agmantine (fragment of Agm lower mass ion). The highest peak on the spectrum (*m/z* 690) refers to the molecular ion after the loss of Cl- group from the end position (Cl^-_{MW}=36). Also, Xyl can be easily detached from the molecule, forming a new lower mass fragment. This can be seen on mass spectra below, where a mass loss of 132 is observed between 726.6 and a peak at 594. Other fragments also verified the given structure as shown on Figure 1.

Table 2. Oligopeptides detected in Polemidia Dam during 2017-2018.

Class	*m/z*	Sequence
Aeruginosins	726.6	Cl-Pla-Leu-Choi(-Xyl)-Agm
Aeruginosins	686.5	Hpla-Tyr-Choi-Angol
Anabaenopeptins	880.2	Ile-CO-Lys-[Ile-Hph-*N*MeHty-AcSer]

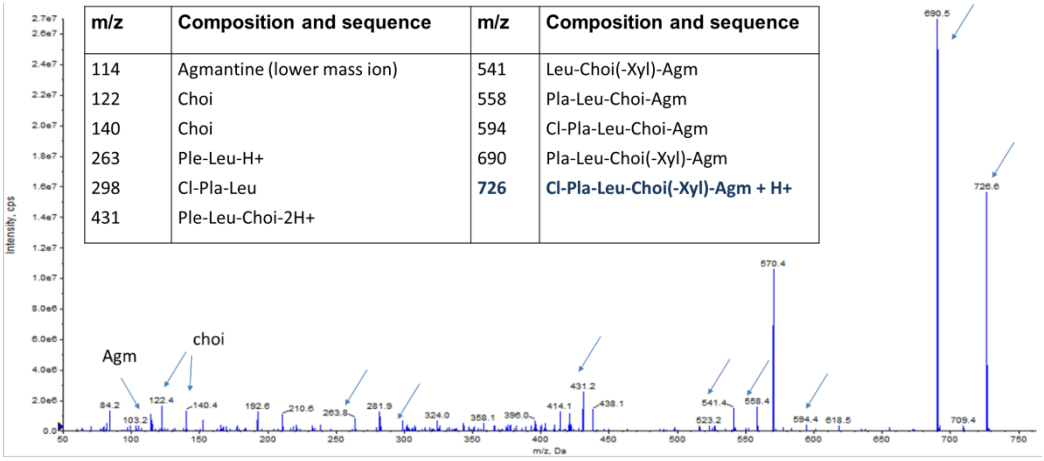

Figure 1. The MS/MS spectrum of the *m/z* 726.6 (RT 7.67min) aeruginosin present in sample (December 2017) and identification of the product ions observed.

4. Conclusions

The oligopeptides detected in Polemidia Dam throughout the years, belong to the classes of microginin, aeruginosins, microcystins and anabaenopeptins. All these years a variety of oligopeptides with different structures and classes have been present in the water samples. Since these oligopeptides are of great concern due to their toxicity and biological activity, it is advised, that toxicity assessment of bloom events should include the presence of all these bioactive compounds.

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