Non-ribosomal cyanopeptides founded in Brazilian Cyanobacteria Bloom: Identification and characterization by MALDI-TOF-MS.

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Abstract

Cyanobacterial bloom is a common problem in the world, there are many species that produce a huge variability of secondary metabolites, mostly oligopeptides that can be toxic or that present bioactivity. In Brazil, there is a harmful bloom of cyanobacteria in Americana, State of São Paulo. In this bloom, many kinds of non-ribosomal cyanopeptides, such as aeruginosins, cyanopeptolin and different microcystins, can be identified using MALDI-TOF-MS.

Introduction

Cyanobacteria are photosynthetic and prokaryote microorganisms that have lived on Earth for billions of years.[1] Their survival may be explained, in parts, by the enormous diversity of the secondary metabolites. Most of them are oligopeptides, called cyanopeptides, which have been ranked into seven major classes: aeruginosins, cyanopeptolins, anabaenopeptins, microginins, microviridins, ciclamides, microcystins.[2] Most of these compounds are produced by non-ribosomal pathways, with the exception of ciclamides and microviridins.[3]

An efficient technique for the analysis and characterization of different cyanopeptides present in cyanobacteria's blooms is the Matrix-Assisted Laser Desorption Ionization (MALDI-MS).[5] This technique is extremely fast, has high resolution, does not require sample treatment, has no interference in the analysis and has low reagent consumption. For these reasons, MALDI-MS has been widely used in the detection of various types of cyanopeptides because it has high detectability and only needs small quantities of sample.[6] Furthermore, it is able to directly identify structures of new congeners in environmental samples without the need to isolate these substances or laboratory cultivation of cyanobacteria. For these many advantages, we used MALDI-TOF-MS analysis in this study for the identification and characterization of various oligopeptides produced by cyanobacteria and different types of oligopeptides detected in a toxic bloom, in Americana, São Paulo, Brazil.

Results and Discussion

In the cyanobacterial bloom samples collected in Americana were detected *m/z* ion signals referring to numerous variants of microcystins, aeruginosins and cyanopeptolins. The Table 1 shows the *m/z* ion signals and their respective cyanopeptides. The structure of each was confirmed by experiments of high-resolution analysis by MALDI-TOF-MS.

Table 1. Cyanopeptides that were identified in Americana- SP, Brazil.

Cyanopeptides	Theoretical *m/z* $[M + H]^+$	MALDI *m/z* $[M+H]^+$	MALDI-MS Error(ppm)
Aeruginosin 602	603.35007	603.3510	1.54
Aeruginosin 298A	605.36572	605.3663	0.95
Aeruginosin 644	645.36064	645.3586	-3.16
Aeruginosin 646	647.37620	647.3776	2.03
Cyanopeptolin 972	973.53530	973.5425	7.39
Cyanopeptolin986A	987.55096	987.5488	-2.18
MC-LR	995.55604	995.5626	6.59
MC-HilR	1009.5300	1009.5429	7.52
MC-RR	1038.5730	1038.5757	2.51
MC-YR	1045.5353	1045.5437	8.03
Cyanopeptolin 1071	1072*	1072.6240	--
Microviridin 1707	1707.75*	1707.6777	--

Conclusions

In the samples collected from the cyanobacterial bloom were identified eleven non-ribosonal peptides: five congeners of hepatotoxic microcystins and eight protease inhibitors, being four aeruginosins, three cyanopeptolins and one ribosomal peptide, microviridin 1707.

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[1]Metcalf, J. S., e G. A. Codd, *Nova Science*,**2009**,1. [2] Welker, M., e H. von Dohren. *FEMS Microbiol Rev*,**2006**, *30*, 530-63. [3] Neilan, B. A., E. Dittmann, L. Rouhiainen, R. A. Bass, V. Schaub, K. Sivonen, e T. Borner. *J Bacteriol*, **1999**,*181*, 4089-97. [5] Howard, K. L., e G. L. Boyer. *Analytical Chemistry*,**2007**,*79*,5980-5986. [6]Agha, R., e A. Quesada. *Toxins (Basel)*, **2014**, *6*, 1929-50.