EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

CYANOCOST – ES 1105 Action

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Cyanobacterial blooms and toxins in water resources: Occurrence, impacts and management

Short Term Scientific Mission (STSM)

Cyanotoxins and other non-ribosomal peptides in cyanobacteria populations of the shallow hypertrophic lake

Objectives

The aim of the STSM is to analyze spatial and temporal variations of cyanotoxins and other non-ribosomal peptides in hypertrophic shallow lake and to determine possible toxin producers.

Methodology

Sampling was performed in the shallow hypertrophic lake. Samples were collected monthly at 1 m vertical intervals in the deepest part of the lake from May to October in 2015. Lake water samples for cyanotoxins analysis were passed through GF/F filters. In addition, 79 strains of 14 potentially toxic cyanobacteria species were isolated from the lake and grown in MWC medium, the obtained biomass was concentrated by centrifugation and lyophilized.

Cyanotoxins and related peptides analysis. Microcystins (MCs), anatoxin-a (ANTX-a) and cylindrospermopsin (CYN) were extracted with 75% methanol. For the extraction of saxitoxins (STXs), a mixture containing 4 mM ammonium formate buffer (pH 3.5) and acetonitrile (95:5, v/v), at a ratio of 2:3 was used. Five field samples and 23 cyanobacteria isolates were selected for quantitative analysis of CYN, MCs, ANTX-a, STXs. Cyanopeptides and cyanotoxins were analysed using the LC–MS/MS system according to Grabowska & Mazur-Marzec (2011) and Grabowska et al. (2014).

Molecular analysis. Twenty eight strains of 11 cyanobacteria species were selected for detection of microcystin synthetase gene (*mcyE*). Genomic DNA was extracted using Fast DNATM Spin Kit. Specific primers and PCR conditions were used as described in Jungblut & Neilan (2006).

Results

Five classes of cyanopeptides: microcystins, aeruginosins, anabaenopeptins, cyanopeptolins and microginins were detected in the field cyanobacteria samples and the cyanobacteria isolates (Table 1). Representatives of aeruginosins and cyanopeptolins were the most common metabolites detected in the samples. A great variety of peptides in *Planktothrix agardhii*, *Microcystis viridis*, *M. wesenbergii* and *M. aeruginosa* strains were found. Seven MCs variants were identified in the strains of *M. viridis*.

Three MCs variants (MC-RR, MC-LR and MC-YR) were determined in the field samples. The highest total concentration of MCs was assigned in July and September. *M. viridis* and *P. agardhii* were confirmed as MCs producers. ANTX-a was detected only in the field samples. The highest concentrations were found in July and August. STXs were determined in the samples collected in July-August and only *Aphanizomenon gracile* was considered to be an effective producer of these toxins.

The molecular analysis using conventional PCR detected MC synthetase gene (*mcyE*) in *M. viridis* strains.

Table 1. Cyanopeptides classes detected in the samples

Samples	Micro-cystins	Anabae-napeptins	Aerugi-nosins	Cyano-peptolins	Micro-ginins	Unknown
Species						
Microcystis aeruginosa	-	x	x	x	x	x
Microcystis cf. *botrys*	-	-	x	x	-	x
Microcystis flos-aquae	-	x	-	-	-	x
Microcystis wesenbergii	-	-	x	x	-	x
Microcystis viridis	x	-	-	x	-	x
Aphanizomenon gracile	-	x	-	-	-	x
Cuspidothrix issatschenkoi	-	-	-	-	-	-
Sphaerospermopsis aphanizomenoides	-	x	-	-	-	-
Dolichospermum planctonicum	-	-	-	-	-	-
Anabaenopsis cf. *ellenkinii*	-	-	-	-	-	-
Limnothrix planctonica	-	-	-	-	-	-
Planktothrix agardhii	x	x	x	x	-	x
Pseudanabaena limnetica	-	-	x	-	-	x
Planktolyngbya limnetica	-	-	-	-	-	x
Field samples (cyanobacteria biomass on GF/F filters)						
May	-	-	-	-	-	x
June	-	-	-	-	-	x
July	-	x	-	-	x	x
August	-	x	x	-	-	x
September	-	x	x	x	-	x
October	-	x	-	x	-	x

Highlights

- Cyanobacteria populations especially *Planktothrix agardhii*, *Microcystis viridis*, *M. wesenbergii* and *M. aeruginosa* from the studied lake were rich in cyanopeptides.
- *Aphanizomenon gracile* was responsible for saxitoxins synthesis in the lake. *Microcystis viridis* and *Planktothrix agardhii* strains have been shown to produce microcystins.
- *Microcystis viridis* synthesized a great variety of microcystins variants.

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COST is supported by the EU RTD Framework Programme

EUROPEAN SCIENCE FOUNDATION
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ESF provides the COST Office through a European Commission contract