



CYANOCOST – ES 1105 Action

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

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Researcher

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Short Term Scientific Mission (STSM)

On the way to develop molecular methods for the monitoring of emerging cylindrospermopsin (CYN) producers in Europe

Objectives

To develop a **molecular method** for the detection of **CYN-producing *Anabaena*** in field samples, based on selective amplification CYN biosynthesis genes (*cyrA*, *cyrB*, *cyrC* and *cyrJ*). The STSM focused on optimizing of the method in cyanobacterial cultures, making it available for future tests with water samples potentially containing CYN –producing *Anabaena* spp.

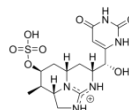


Fig.1. Cylindrospermopsin



Short bio

- ✓ **2012-present:** *postdoctoral fellow*. CH₄ and CO₂ remediation and biofuels from cyanobacteria. JCU University, Australia.
- ✓ **2006-2012:** *PhD Microbiology*. Phylogeny, ecophysiology & annual cycles of toxic cyanobacteria. Autonoma de Madrid University, Spain.
- ✓ **2008:** *collaborator* Nostotox project. Dr. Claudia Wiedner's group. IGB, Germany.

Methodology

- 1. PCR and sequencing of partial *cyrA*, *cyrB*, *cyrC* and *cyrJ* genes in CYN-producing *Anabaena*:** primer design (NetPrimer and Primer 3) and PCR optimization (gradient PCR) for conserved regions between 468bp (*cyrC*) and 859bp (*cyrA*), tested with positive (CYN-producing *A. lapponica*, *Aph. ovalisporum* and *Oscillatoria* sp.) and negative controls (non-producing *Anabaena* sp. and *A. planctonica*).
- 2. Design of *A. lapponica*-specific primers:** design of *A. lapponica*-specific primers targeting a 225bp *cyrB* fragment (suitable for qPCR), tested in positive and negative controls.
- 3. Phylogeny of CYN-producing *A. lapponica*:** phylogenetic trees (MEGA 5.0) based on newly obtained *A. lapponica* sequences of housekeeping genes (16S rRNA and *rpoC1*) and CYN biosynthesis genes (*cyrA*, *cyrB*, *cyrC*, *cyrJ*).

Results

Design of general primers (*cyr* genes)

Gene	Primer	Amplicon length (bp)	Reference	Use
<i>cyrA</i>	aoaFa	859	This study	General PCR, sequencing
	aoaRa		This study	
<i>cyrB</i>	cyrB2137F	576	This study	General PCR, sequencing
	cyrB2851R		This study	
	cyrB2600F	224	This study	<i>A. lapponica</i> -specific PCR
<i>cyrC</i>	cyrC2824R		This study	
	A205PKF	468	[1]	General PCR, sequencing
<i>cyrJ</i>	A205PKR		[1]	
	cyrJ48F	502	[2]	PCR, sequencing
	cyrJ644R		This study	

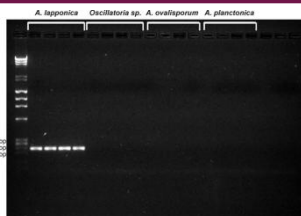
Table 1. Primers used in this study.
[1] Kellman et al. 2006. [2] Mihali et al. (2008)

Strain	CYN	<i>cyrA</i>	<i>cyrB</i>	<i>cyrC</i>	<i>cyrJ</i>
<i>An. lapponica</i> Syke966	+	+	+	+	+
<i>An. planctonica</i> UAM516	-	-	-	-	-
<i>Anabaena</i> sp. Anab202A1	-	-	-	-	-
<i>Aph. ovalisporum</i> UAM290	+	+	+	+	+
<i>Oscillatoria</i> sp. PCC6506	+	+	+	+	+

Table 2. Primer tests with strains

A. lapponica specific primers (*cyrB*)

Fig.2 Agarose gel showing species-specific amplification of *cyrB* in *A. lapponica* (gradient PCR)



Phylogeny of *cyr* genes in *A. lapponica*

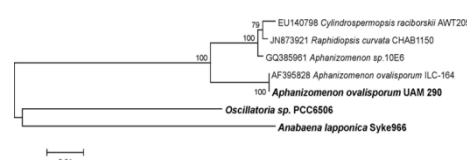


Fig 3. NJ tree of concatenated *cyrA*, *cyrB*, *cyrC* and *cyrJ* (2137 bp). Strains from this study marked in bold.

Highlights

- ✓ First study sequencing 16S rRNA, *rpoC1*, *cyrA*, *cyrB*, *cyrC* and *cyrJ* in *A. lapponica*
- ✓ Design of *A. lapponica* - specific primers targeting a *cyrB* fragment (225 bp), suitable for qPCR
- ✓ Unexpected phylogeny of *cyr* genes in *A. lapponica*, more related to benthic producers

Host Organization

Dr. Kaarina Sivonen's group
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