



**Integration of PAper-based Nucleic acid testing mEthods
into Microfluidic devices for improved biosensing Applications**

Research on cyanobacteria and cyanotoxins at the Faculty of Sciences—
detection methods

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IPANEMA, University of Maribor, July 24 2025, Maribor



This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 872662

- One of the largest universities in Central Europe
 - **2nd largest university in Serbia, founded in 1960**
 - **14 faculties**
 - **50.749 students**
 - **5.000** professors, assistants, researchers and supporting staff
 - **66** foreign teaching staff and associates
 - **390** accredited study programmes
 - **Science:** leading position in the region; research projects, accredited Centers of Excellence
 - **Third Mission: responsible knowledge society**
- 140** start-up and spin-off companies in the field of ICT;
Novi Sad – *Serbian Silicon Valley*;

Comprises faculties located in the 4 major cities in Vojvodina province:



- **UNSPMF was founded in 1969.**
- It is a public, governmental, and non-for-profit institution, fully committed to education, teaching and research
- The Faculty employs 615 staff and educates more than 5000 students
- It is comprised of **5 departments**:
 - **Biology , Ecology , Molecular Biology, Bioinformatics**
 - **Chemistry, Biochemistry and Environmental Protection**
 - **Mathematics and Informatics**
 - **Geography, Tourism and Hotel Management**
 - **Physics**
- **The three-cycle system of academic studies: undergraduate, master and doctoral studies with more than 50 study programs**
- The Faculty developed an internal strategy for enhancing the educational and research processes, quality assurance mechanisms, and internationalization, which heavily rely on capacity building, interactive networking, international scientific projects, academic mobility at all levels, and visibility on a global scale; more than **60 projects are ongoing**; bi- and multilateral cooperation with more than 50 universities and institutes worldwide (Thailand, Australia, USA, Germany, Austria, Switzerland, Slovenia, Croatia, France, Portugal, Italy, Finland, Spain, Belarus, etc.).



There are two Centers of Excellence:

[Centre of Excellence in Mathematical Research of Non-linear Phenomena](#)

The activity of the Centre is mainly scientific and it comprises three research groups at the Department of Mathematics and Informatics:

Group for analysis, probability and geometry,

Group for numerical mathematics and

Group for model-theoretic forcing and set-theoretic logic

[Centre for Reproductive Endocrinology and Signalling \(CeRES\)](#)

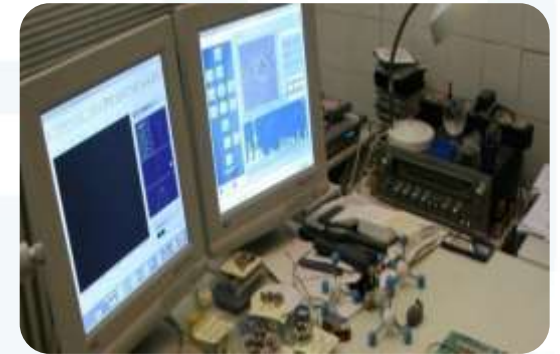
The activity of the Centre is mainly scientific and comprises the work of one research laboratory (at the Department of Biology and Ecology) and three research groups (two from the Department of Biology and Ecology and one from the Department of Mathematics and Informatics) whose competences are complementary:

[LaRES \(Reproductive Endocrinology and Signalling\)](#)

SiEB (Signalling in Evolutionary Biology)

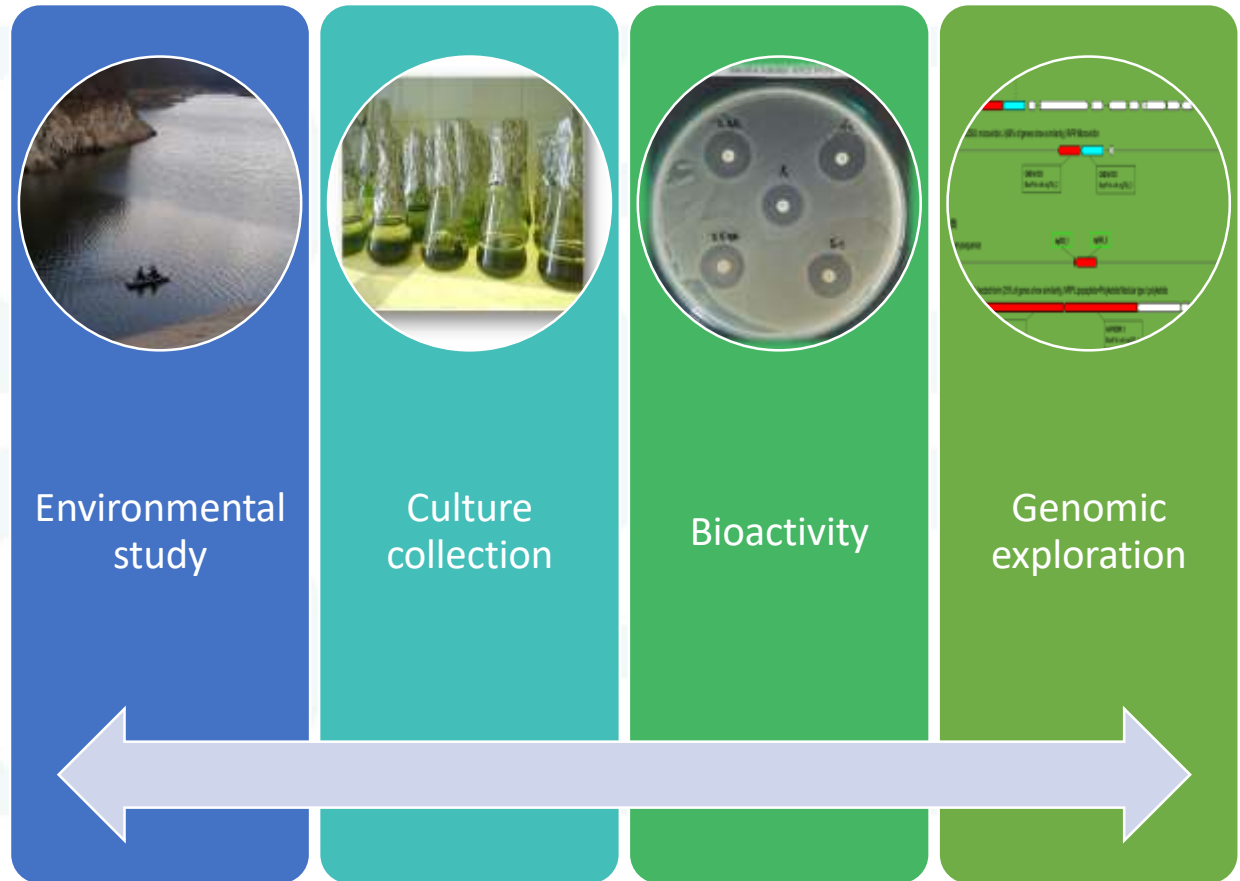
SiDM (Signalling in Discrete Mathematics)

SiSMB (Signalling in Structural and Molecular Biology)



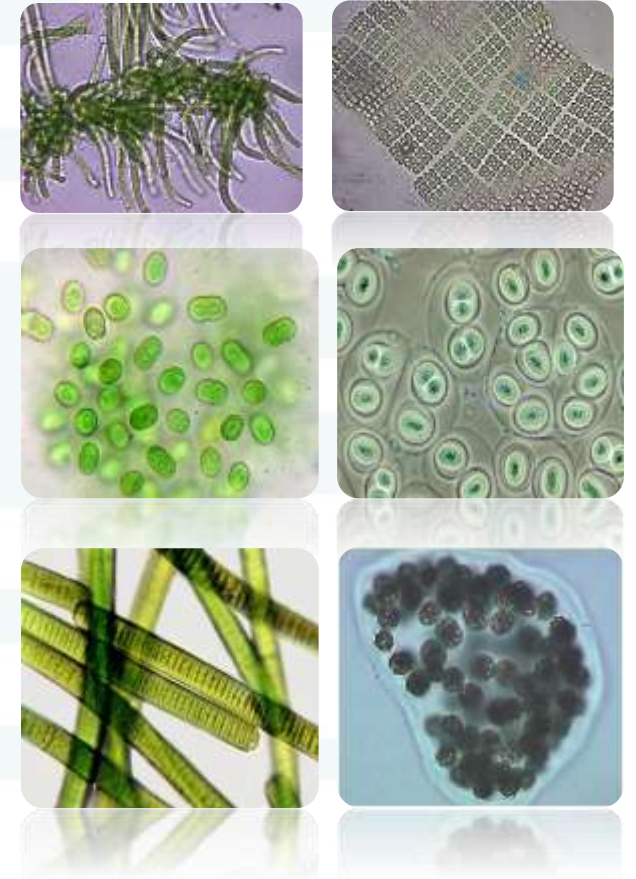
Cyanobacteria

Green microalgae



WHAT ARE CYANOBACTERIA?

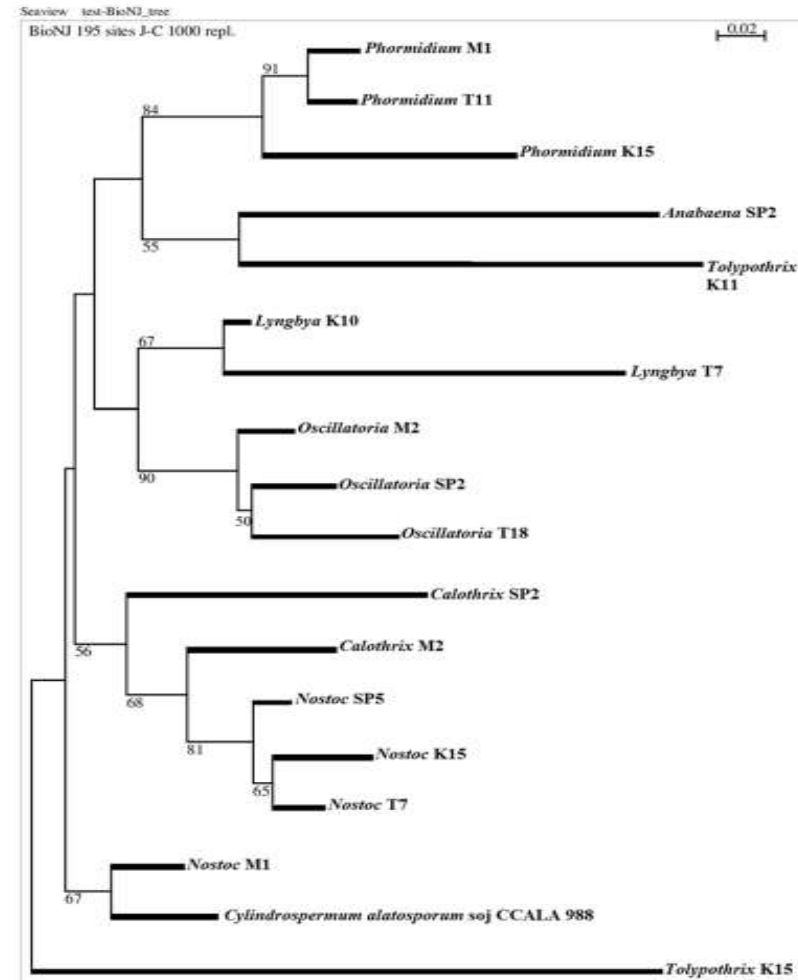
- **The oldest oxygenic photoautotrophs** - extremely diverse group of microorganisms - key role in almost every ecosystem
- **Formerly known as blue-green algae** (they are more closely related to procariotic bacteria than eucariotic algae)
- **Extremely adaptable** - can be found in almost any environment including unfavorable conditions
- **High metabolic potential** - they have a superior ability - they change their modes of nutrition
- **Ecologically and economically important** - the most promising group in biotech applications - sources of useful natural products
- **Can be toxic - hundreds of toxins**



CULTURE COLLECTION at UNSPMF

Novi Sad Cyanobacterial Culture Collection

- Isolation, purification and cultivation of strains
- Identification and phylogenetic analysis (16S rRNA)
- Ecophysiological characterization
- Bioactivity and biochemical characterization
- Toxicity testing**

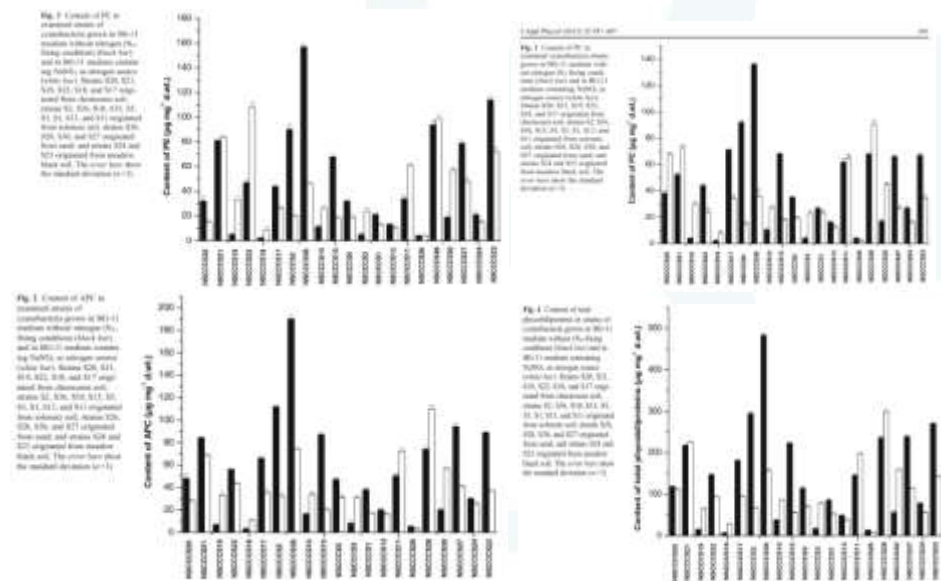
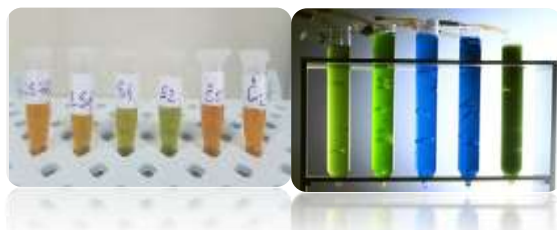


1) Ecophysiological and biochemical characterization

1. Production of biomass

2. Production of pigments (natural colorants):

- ✓ Phycocyanin
- ✓ Allophycocyanin
- ✓ Phycoerythrin
- ✓ Chlorophyll



Simeunović et al. 2013 JAPH

THE PRODUCTION OF BIOMASS AND PHYCOBILIPROTEIN PIGMENTS

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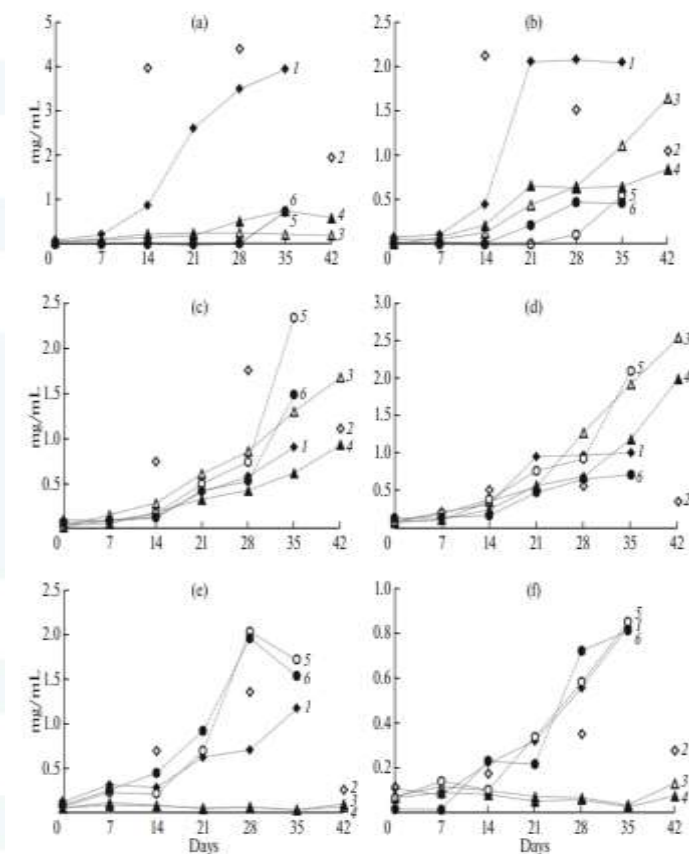


Fig. 1. Biomass production in different cyanobacteria. (a) Spirulina S1, (b) Spirulina S2, (c) Anabaena C2, (d) Anabaena C3, (e) Nostoc 2S7B, (f) Nostoc 2S9B. 1—Control; 2—continuous illumination; 3—0.15% glucose; 4—0.3% glucose; 5—0.15% glycerol; 6—0.3% glycerol.

Blagojević et al. 2017 AMB



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2) Bioactivity and biochemical characterization of cyanobacteria

- Antioxidant activity
- Antimicrobial activity
- **Toxicity**
- Identification of BAM

Table 1 Antioxidant activity of tested cyanobacterial strains (ethanolic extracts) cultivated under diazotrophic (d) and non-diazotrophic (n) conditions

| Cyanobacterial strain | DPPH assay IC ₅₀ (mg mL ⁻¹) | Ferric reducing/antioxidant power (FRAP) assay (mg AAE g ⁻¹) |
|---------------------------------|---|---|
| <i>Nostoc</i> 2S7B ^d | 0.11 ± 0.005a | 12.23 ± 0.13c |
| <i>Nostoc</i> 2S7B ⁿ | 0.11 ± 0.001a | 13.28 ± 0.29c |
| <i>Nostoc</i> 2S9B ^d | 4.71 ± 0.59b | 10.22 ± 0.97d |
| <i>Nostoc</i> 2S9B ⁿ | 0.13 ± 0.004a | 9.13 ± 0.34d |
| <i>Nostoc</i> 2S1 ^d | 1.39 ± 0.63a | 13.69 ± 1.62c |
| <i>Nostoc</i> 2S1 ⁿ | 1.76 ± 0.26a | 11.92 ± 0.41c |
| <i>Nostoc</i> 2S3B ^d | 9.47 ± 3.61b | 9.44 ± 0.28d |
| <i>Nostoc</i> 2S3B ⁿ | 5.84 ± 2.26b | 8.36 ± 0.08d |
| <i>Nostoc</i> S8 ^d | 0.06 ± 0.01a | 11.34 ± 0.68c |
| <i>Nostoc</i> S8 ⁿ | 0.50 ± 0.12a | 11.20 ± 0.38c |
| <i>Nostoc</i> LC1B ^d | 0.04 ± 0.01a | 11.38 ± 0.91c |
| <i>Nostoc</i> LC1B ⁿ | 2.11 ± 0.70a | 11.53 ± 0.47c |
| <i>Anabaena</i> C2 ^d | 0.11 ± 0.01a | 12.30 ± 0.35c |
| <i>Anabaena</i> C2 ⁿ | 0.09 ± 0.003a | 12.64 ± 0.40c |
| <i>Anabaena</i> C5 ^d | 0.12 ± 0.01a | 12.89 ± 0.43c |
| <i>Anabaena</i> C5 ⁿ | 0.12 ± 0.01a | 11.38 ± 0.20c |
| <i>Arthrospira</i> S1 | 0.12 ± 0.004a | 21.01 ± 1.66a |
| <i>Arthrospira</i> S2 | 0.10 ± 0.005a | 15.14 ± 0.12b |

Values represent averages ± standard deviations for triplicate experiments. Values in the same column with different lowercase letters are significantly ($P < 0.05$) different

Blagojević et al. 2018 J Appl Phycol

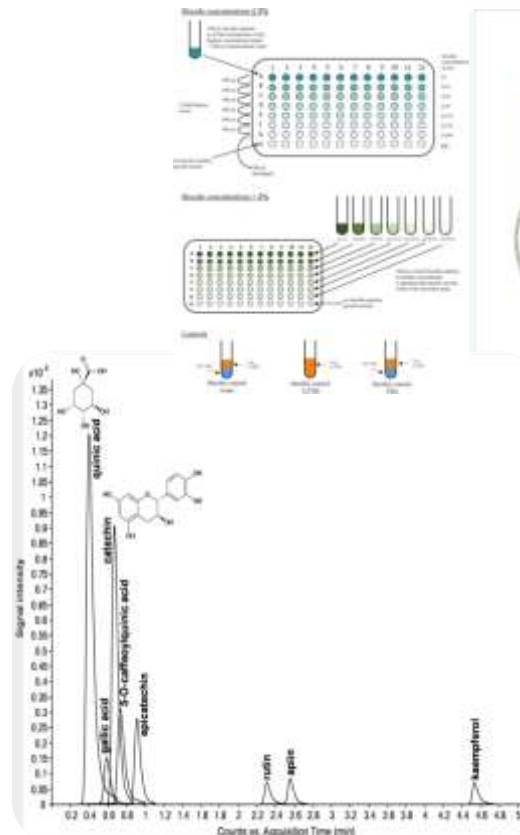
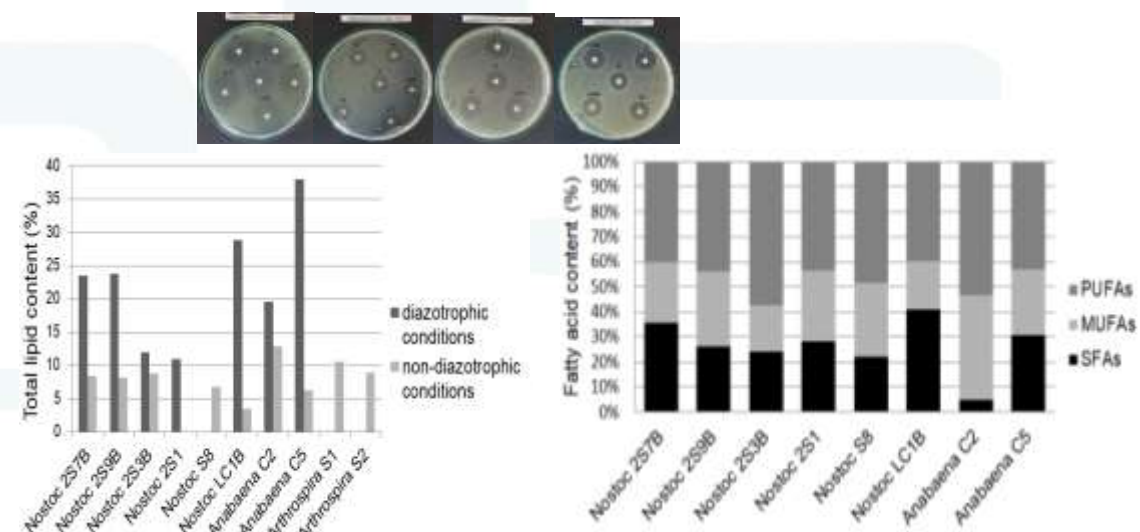
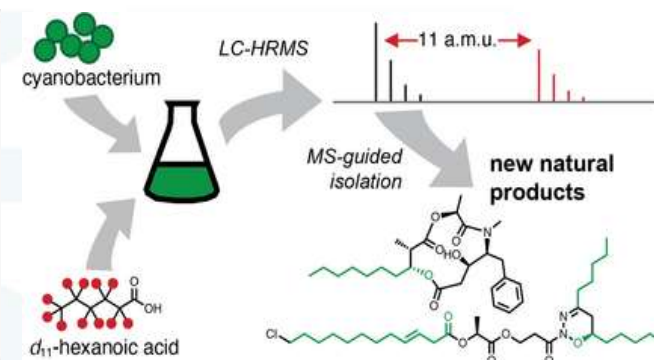
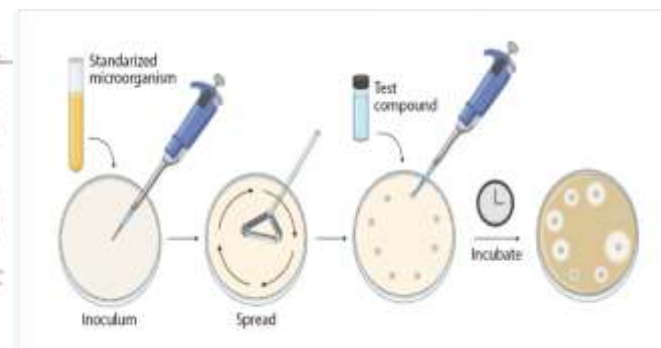


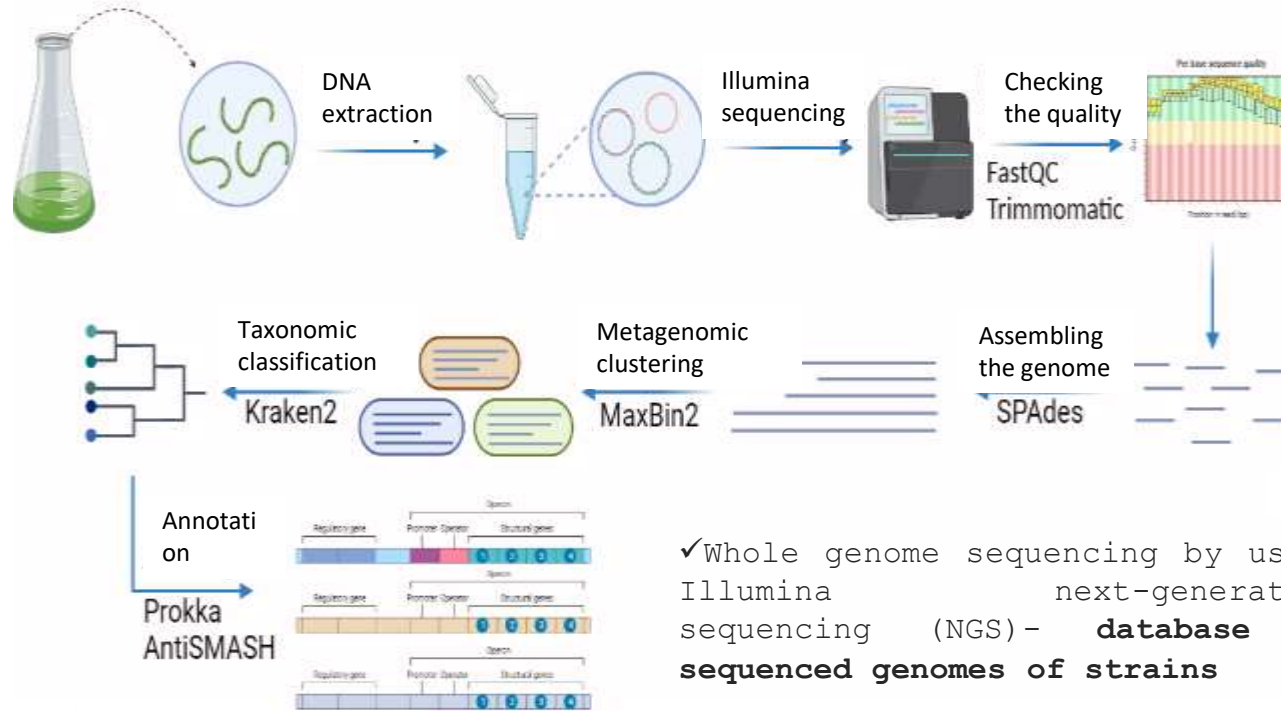
Fig. 1 LC-MS MRM chromatogram of compounds quantified in the examined extracts



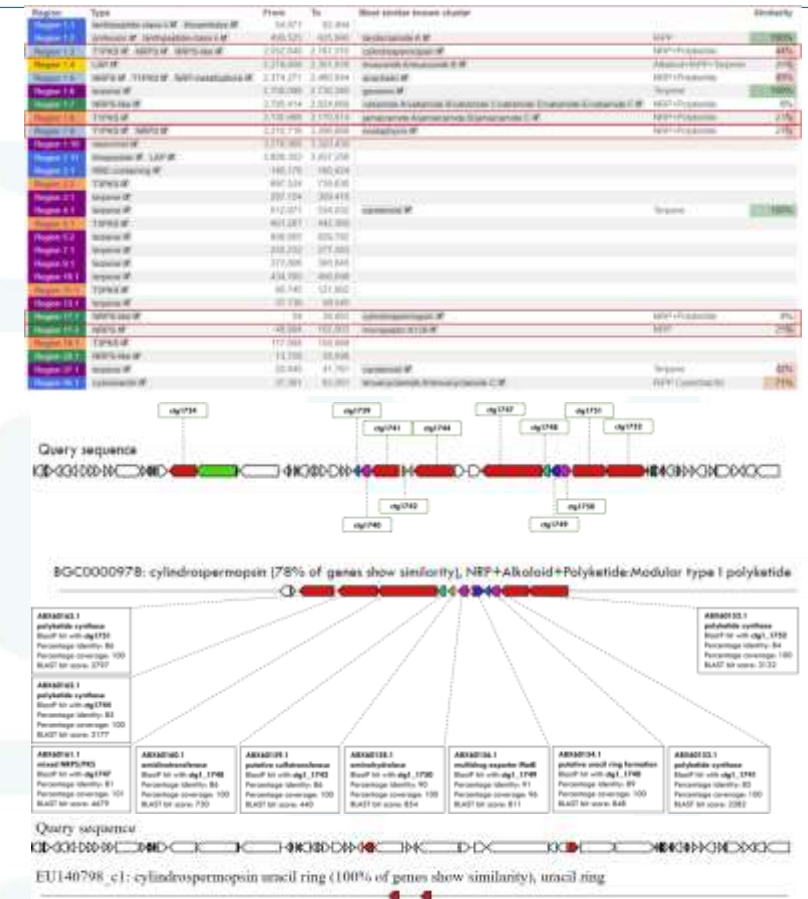
Unpublished data

3) Genome exploration-potential for BAC production

✓ **Project : Molecular methods for supporting microalgae as biofactories (MORAB) - Science Fund of the Republic of Serbia**



✓Whole genome sequencing by using Illumina next-generation sequencing (NGS) - **database of sequenced genomes of strains**

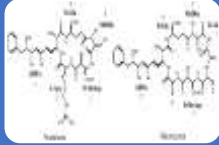


Identification, annotation and analysis of secondary metabolite biosynthesis gene clusters present in the genome of *Oscillatoria nigra-viridis* K3. **A)** presents a summary of all regions for which a match was found in the available databases along with a predicted secondary metabolite product and a degree of similarity (%) to the reference sequence. **B)** presents the best match for the region 1.3, with details for each hit including sequence identity and proposed gene functions

Simeunović et al., 2021; Davidović et al. 2023 Tox SEE

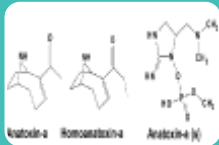
WHAT ARE CYANOTOXINS?

- Secondary metabolites of cyanobacteria - **highly potent toxic compounds**
- Biologically active substances with lethal, sublethal and chronic effects
- **Very common in surface freshwaters during cyanobacterial harmful blooms (CyanoHABs)**
- Heterogeneous group of compounds both by chemical nature and by mode of action



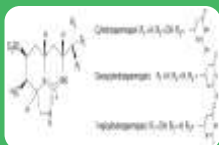
HEPATOTOXINS

- Microcystins
- Nodularins



NEUROTOXINS

- Anatoxins
- Saxitoxins



CYTOTOXINS

- Cylindrospermopsin
- Deoxycylindrospermopsin



DERMATOTOXINS

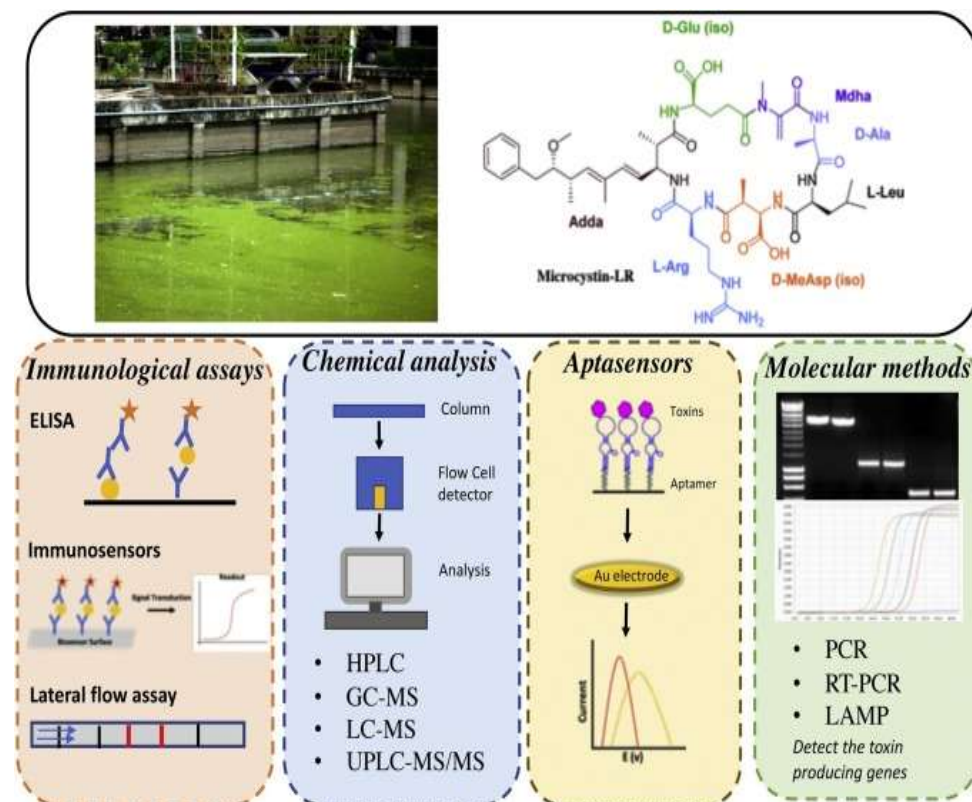
- Aplysiatoxin
- Lyngbyatoxin

Comparison of cyanobacterial toxins with other types of biotoxins (Falconer et al. 1999)

| Toxin | Producers | Lethal dose (LD ₅₀)* |
|-----------------|---|----------------------------------|
| BOTULIN | Clostridium botulinum | 0,00003 |
| TETANUS | Clostridium tetani | 0,0001 |
| RICIN | Ricinus communis | 0,02 |
| DIFTERIA TOKSIN | Corynebacterium diphtheriae | 0,3 |
| KOKI TOKSIN | Phyllobates bicolor | 2,7 |
| TETRODOTOKSIN | Sphaeroides rubripes | 8 |
| SAKSITOKSIN | Aph. flos-aquae (cyanobacterium) | 9 |
| COBRA TOKSIN | Naja naja (kobra) | 20 |
| NODULARIN | Nodularia spumigena (cyanobacterium) | 30-50 |
| MIKROCISTIN-LR | Microcystis aeruginosa (cyanobacterium) | 50 |
| ANATOKSIN-a | Anabaena flos-aquae (cyanobacterium) | 200 |
| MIKROCISTIN-RR | Microcystis aeruginosa (cyanobacterium) | 300-600 |
| CURARE | Chondrodendron tomentosum | 500 |
| STRIHNIN | Strychnos nux-vomica | 500 |
| AMATOKSIN | Amanita phalloides | 600 |
| MUSKARIN | Amanita muscaria | 1100 |
| FALATOKSIN | Amanita phalloides | 1800 |
| GLENODIN TOKSIN | Peridinium polonicum | 2500 |
| CIJANID | | 10000 |

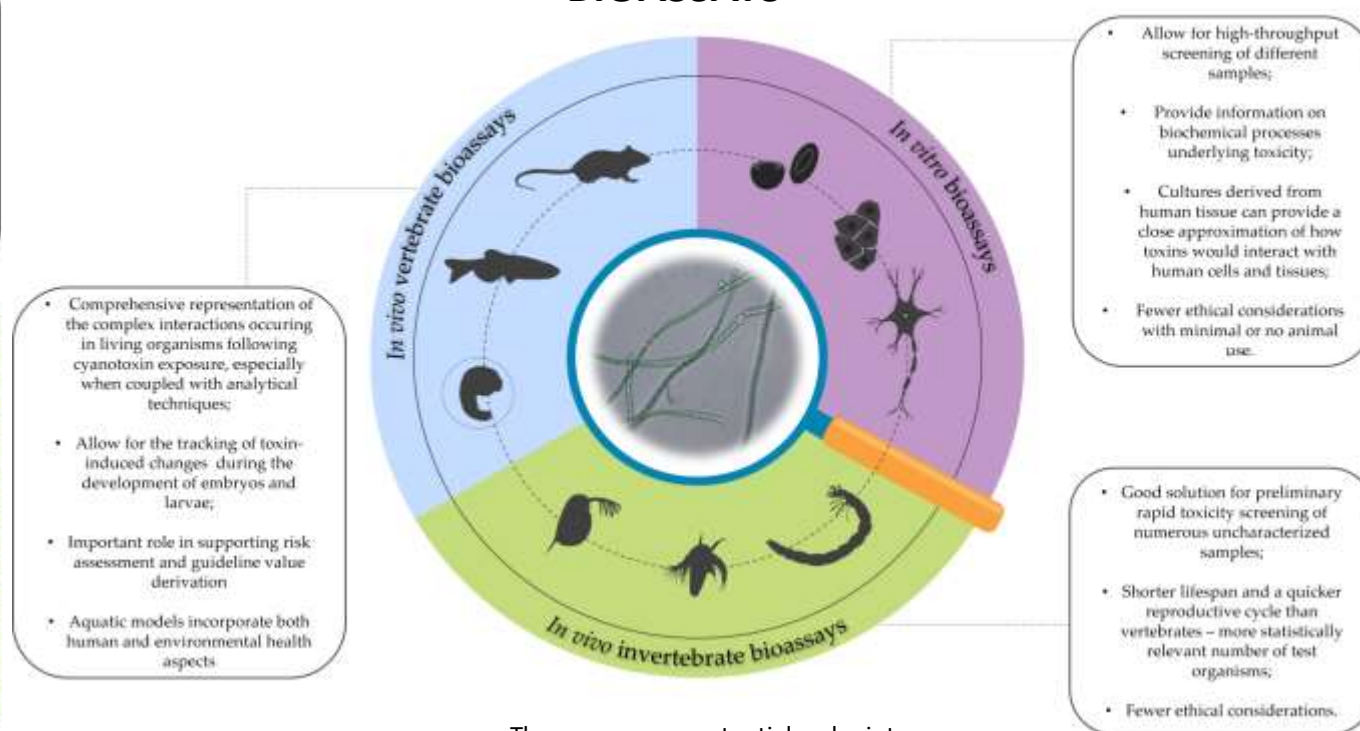
4) METHODS FOR DETECTION AND CHARACTERIZATION OF CYANOTOXICITY

✓ Multi-level approach- a combination of several groups of methods



Kulabhusan et al, 2021 TEACH

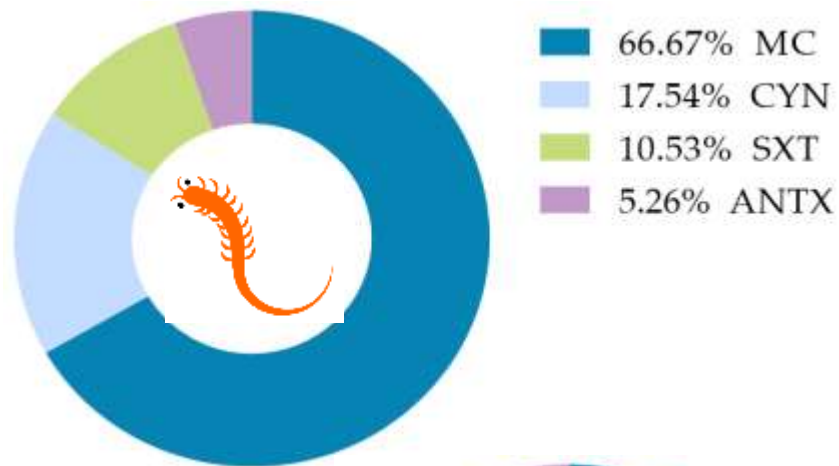
BIOASSAYS



There are many potential endpoints

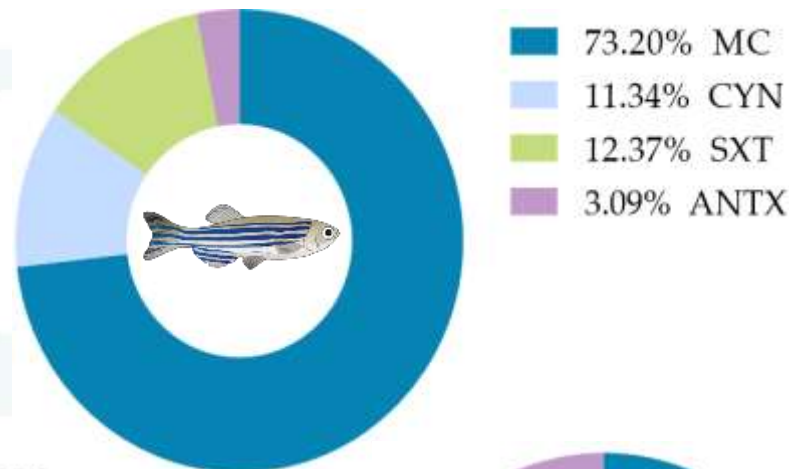
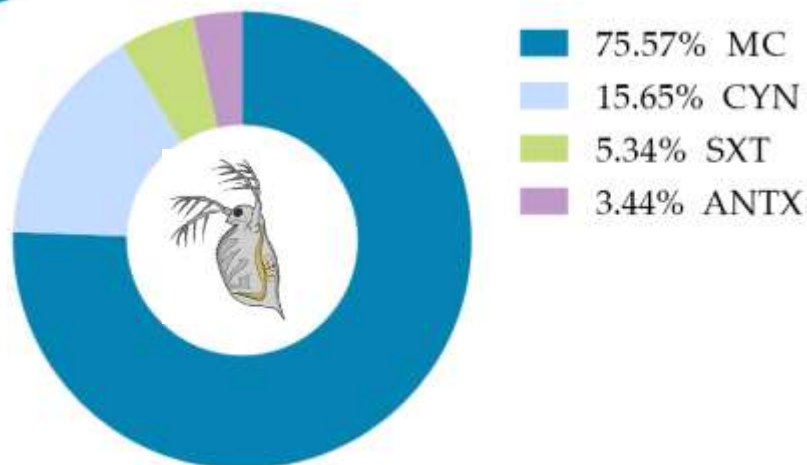
Davidović et al, 2023 Biology

In vivo bioassays used in cyanotoxicity testing



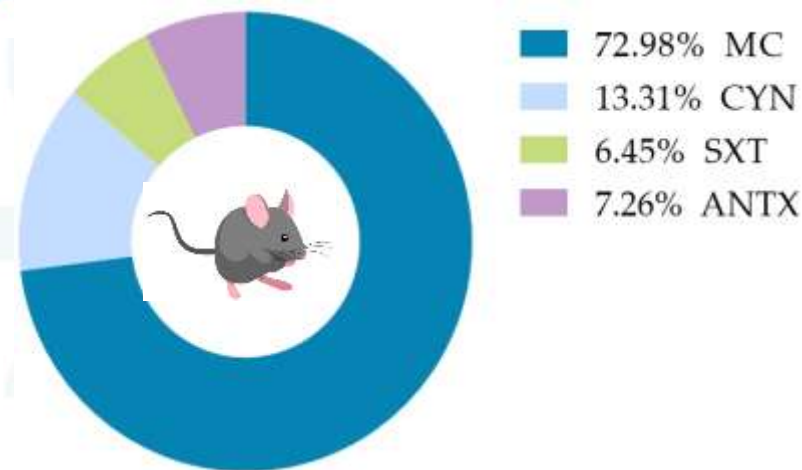
Invertebrate bioassays:

- ✓ Cost effectiveness
- ✓ High throughput screening
- ✓ Valuable insights into the sublethal effects of toxins
- ✓ Fast tests
- ✓ Ease of maintenance
- ✓ Rapid analysis of a large number of samples
- ✓ No or few ethical concern



Vertebrate bioassays:

- ✓ Provide more realistic and comprehensive representation of the effects of cyanotoxins
- ✓ Might help to identify the specific mechanisms by which cyanotoxins cause toxicity
- ✓ Ability to simulate the effects of chronic exposure to cyanotoxins
- ✓ Ethical concern
- ✓ There is interspecies variability in the response to a toxin



Davidović et al, 2023 Biology



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- ✓ Cyanobacterial **toxicity** detection using *in vivo* bioassays
- ✓ The main response criterion is mortality

Acute toxicity

- Bioassay *Artemia salina*
- Kiviranta et al.(1991)

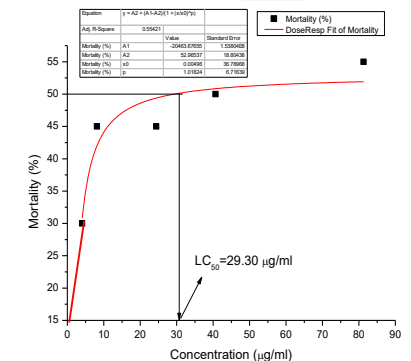
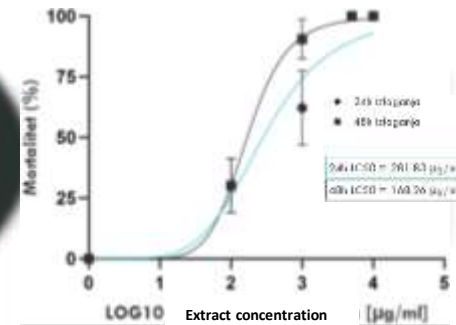
Acute and chronic toxicity

- Bioassay *Daphnia magna*
- ISO (1996)

Acute toxicity

- DarT test- Test with zebrafish embryos (*Danio rerio*)
- Nagel (2002)

✓ SCOPES project IZ73ZO_152274/1 –Swiss National Science Fondation



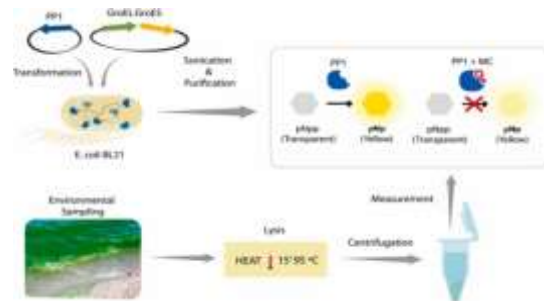
✓Cyanobacterial toxicity detection using *in vitro* bioassays

•**PP1 assay** - Inhibition of the enzyme protein phosphatase 1 (An and Carmichael , 1994)-**hepatotoxicity**

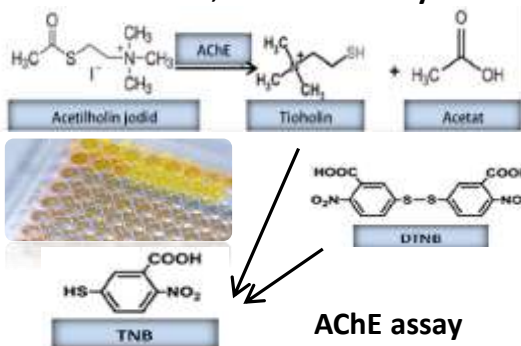
•**AChE assay**—Inhibition of the acetylcholine esterase (Ellman test - Ellman et al. (1961))-**neurotoxicity**

•IC₁₀₀, IC₅₀ and IC₂₅ values

•Positive controls : Microcystin-LR (PP1) i Donepezil (AChE)



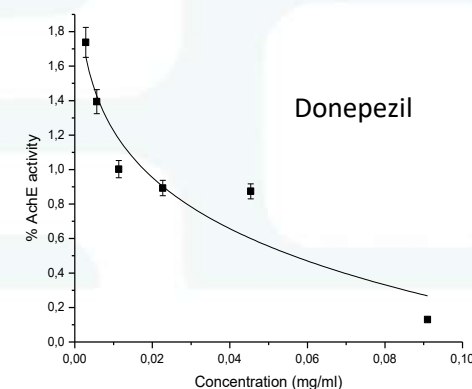
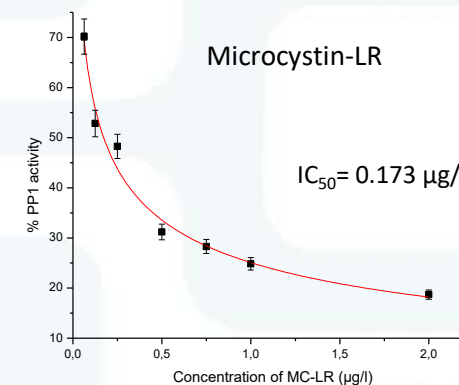
Posse et al., 2023- PP 1 assay



AChE assay

Toxicity of cyanobacterial strains in PP1 and AChE assays

| Tested cyanobacterial strains | Recorded IC values in PP1 assay (mgml ⁻¹) | Recorded IC values in AChE assay (mgml ⁻¹) |
|-------------------------------|---|--|
| Phormidium Z2 | >0.5 | IC ₂₅ =0.073 ±0.356 |
| Microcystis PCC7806 | IC ₅₀ = 0.315±0.114 | IC ₇₅ =0.543 ±0.256 |
| Oscillatoria K3 | IC ₂₅ = 0.258±0.098 | IC ₇₅ =0.025 ±0.293 |
| Nostoc T18 | >0.5 | IC ₁₀₀ =0.49 ±0.019 |
| Nostoc 2S9B | >0.5 | IC ₂₅ =0.50 ± 0.142 |
| Spirulina S2 | >0.5 | IC ₂₅ =1.46 ± 0.106 |
| Nostoc 2S1 | >0.5 | IC ₂₅ =1.82 ± 0.126 |
| Spirulina S1 | >0.5 | IC ₂₅ =1.93 ± 0.132 |
| Anabaena C2 | >0.5 | IC ₂₅ =2.70 ± 0.065 |
| Anabaena C5 | >0.5 | IC ₂₅ =0.58 ± 0.097 |
| Nostoc S8 | IC ₂₅ =0.430±0.212 | >3.00 |
| Nostoc LC1B | IC ₂₅ =0.332±0.148 | >3.00 |



✓ Cyanobacterial toxicity detection using in vitro bioassays

✓ Cell lines



cytotoxicity and hepatotoxicity

human hepatocellular carcinoma cells - **HepG2**

hepatocytes of Rainbow trout (*Oncorhynchus mykiss*) - **RTL-W1**

fish hepatoma cell line derived from topminnow (*Poeciliopsis lucida*) **PLHC-1**



neurotoxicity

human neuroblastoma cell line - **SH-SY5Y**

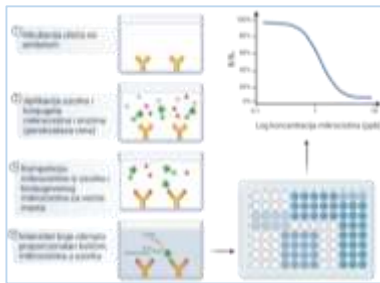


the inhibition of transport activity - Oatp1d1 and Oct1

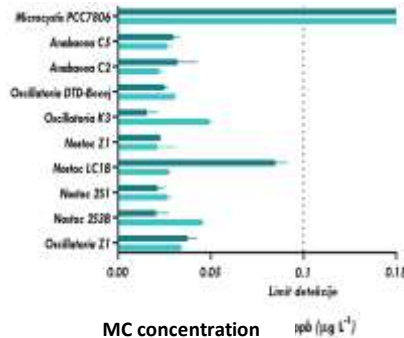
transiently transfected human embryonic kidney cell line- **HEK239**

over expressing two different types of transporters cloned from zebrafish liver

✓ ELISA test- hepatotoxin detection



Davidović et al. 2022 Harmful algae



MC concentration ppb ($\mu\text{g L}^{-1}$)

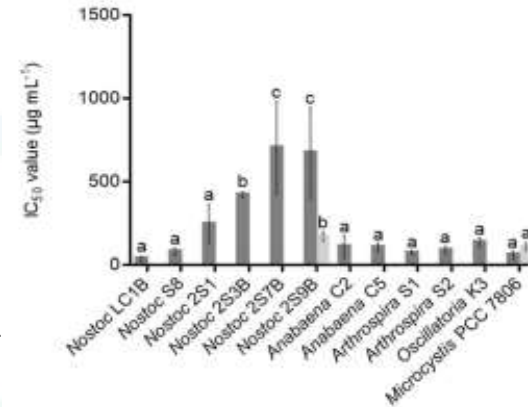
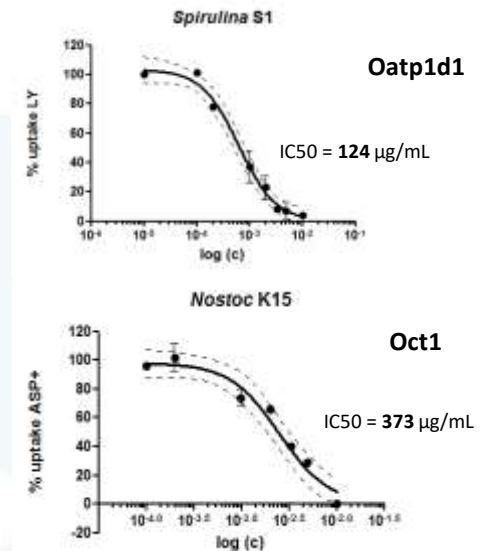
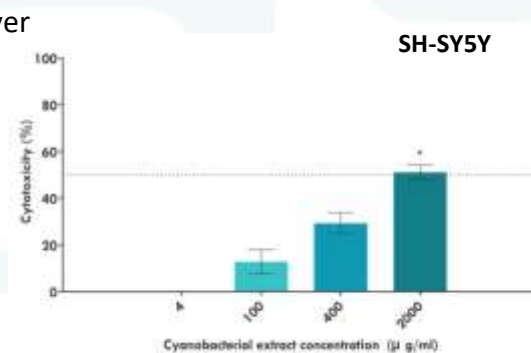


Fig. 1 Cytotoxicity of the tested cyanobacterial extracts to the human hepatocellular carcinoma (HepG2) and rainbow trout (*Oncorhynchus mykiss*) liver-derived (RTL-W1) cells expressed as IC_{50} value. Values represent mean \pm SD. Values in the same cell line with different lowercase letters (^a, ^b, ^c) are significantly different (Tukey HSD test at $p < 0.05$)

Blagojević et al. 2021 Environ Sci Poll Research

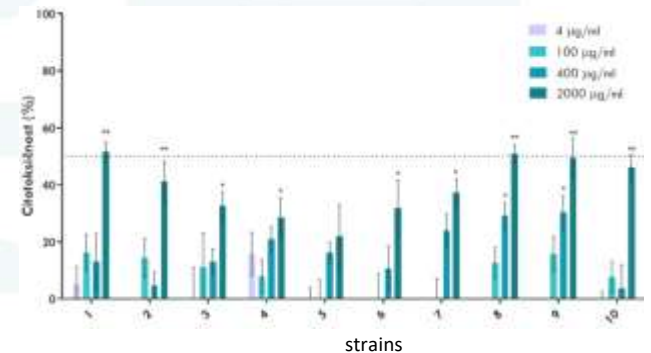


Marić et al. 2020 Ecotoxicology



The effect of cyanobacterial extracts on the viability of SH-SY5Y cells

Davidović et al. 2025 ICTC

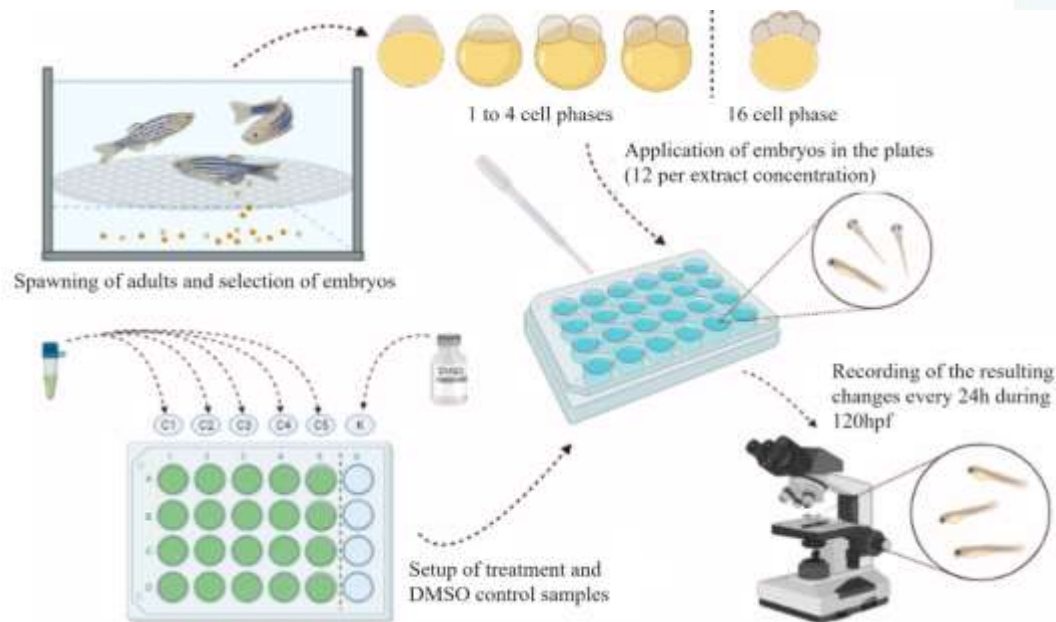


strains

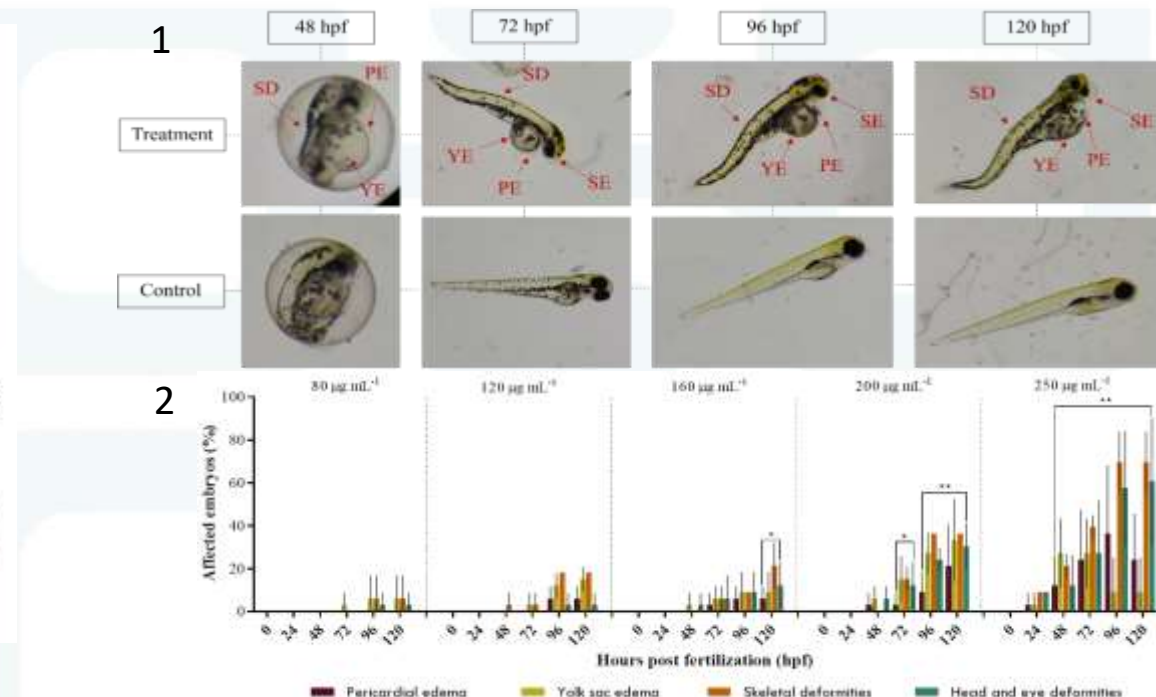


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✓ Embriotoxicity (DarT- zebrafish embryos)



Davidović P. et al. 2025 Harmful Algae

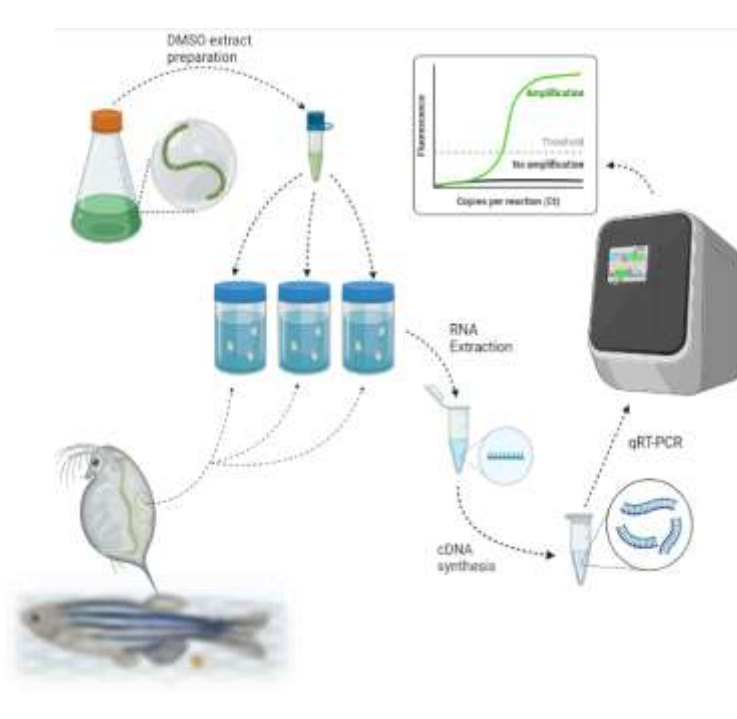


Results of the acute toxicity of the strain OK3 in the bioassay with *D. rerio* embryos.

- 1) Light microscopy images of the observed changes in embryos and larvae in treatment and control group. The most pronounced toxic effects: **PE - pericardial cavity edema, YE - yolk sac edema, SD - skeletal deformity, SE - small eye/reduced eye size**
- 2) Frequency of the 4 most prevalent categories of sublethal changes observed in the treated embryos and larvae.

Davidović P. et al. 2025 Harmful Algae

- ✓ **Genotoxicity** (effects on gene expression in model organisms)
- ✓ Analysis of relative gene expression changes - qRT-PCR



P.G. Davidović et al.

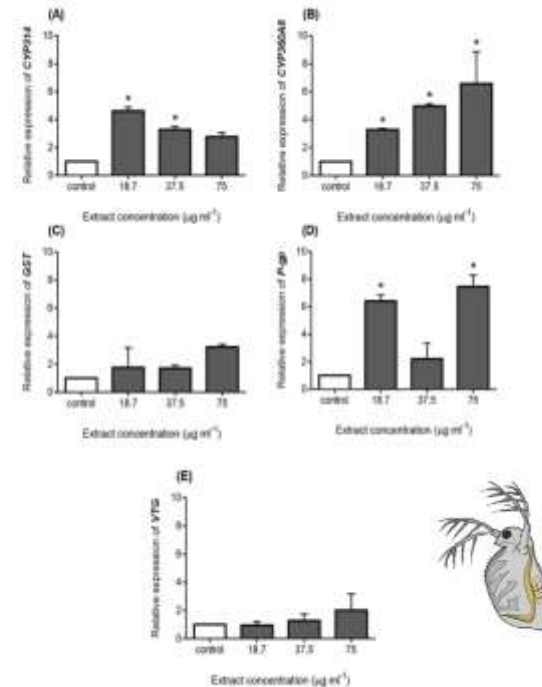


Fig. 2. Effects of three different concentrations of the cyanobacterial extract *Nostoc* Z1 on relative expression levels of five selected genes in *D. magna*: (A) *cyp19a*, (B) *cyp360a*, (C) *gst*, (D) *p-gp*, and (E) *vtg*. Changes in the gene expression levels relative to the unexposed DMSO control groups are presented as columns with mean ± SD values of two independent experiments. Asterisks (*) indicate significant differences between the control and treated groups ($P < 0.05$).

Davidović et al., 2022 Harmful Algae

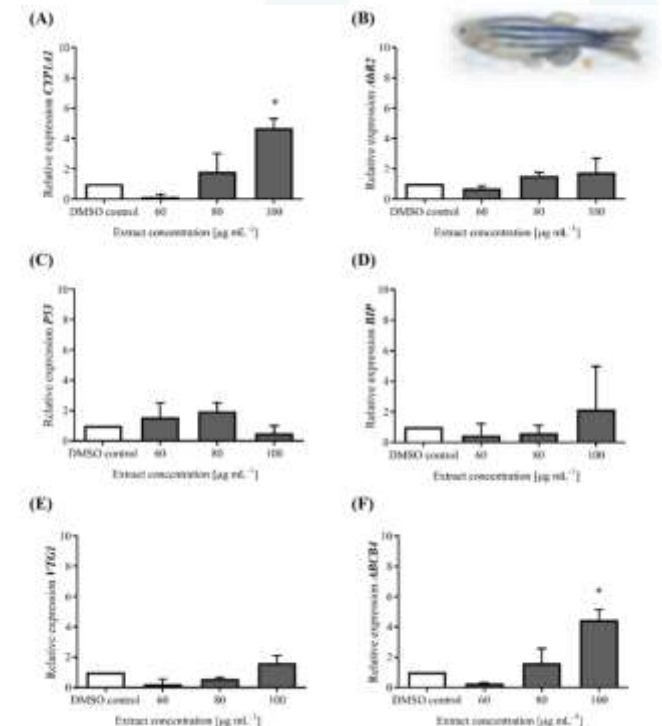


Fig. 3. Effects of the *P. aeruginosa* K3 extract on the relative expression levels of six selected genes in *D. rerio*: (A) *cyp11a*, (B) *ahr2*, (C) *p53*, (D) *bcl2*, (E) *veg* and (F) *ahr1*. Changes in gene expression levels relative to unexposed controls are presented as bars with mean ± SD values of three independent experiments. Asterisks (*) indicate significant differences between control and treated groups ($P < 0.05$).

Davidović et al. 2025 Harmful Algae



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✓ Genome analysis – determining the potential for toxin production

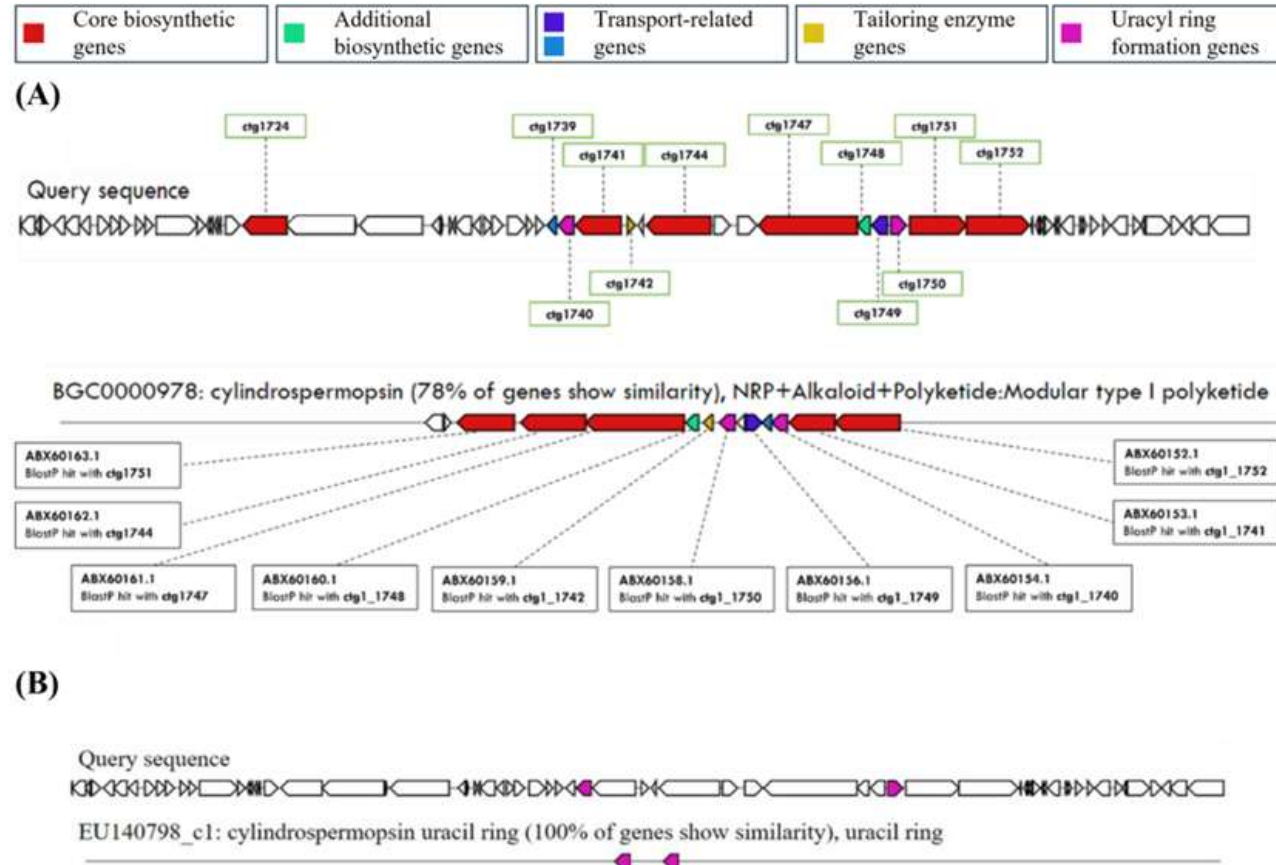


Fig. 4. Alignment of specific regions in the *P. nigroviride* K3 genome with the *Cylandropermopsis raciborskii* AWT205 genome cylandropermopsin biosynthesis gene cluster (A), and the cylandropermopsin uracil ring (B). Genes marked with the same color are interrelated, while white genes have no relationship.

LC-MS/MS determination of toxin cylandropermopsin

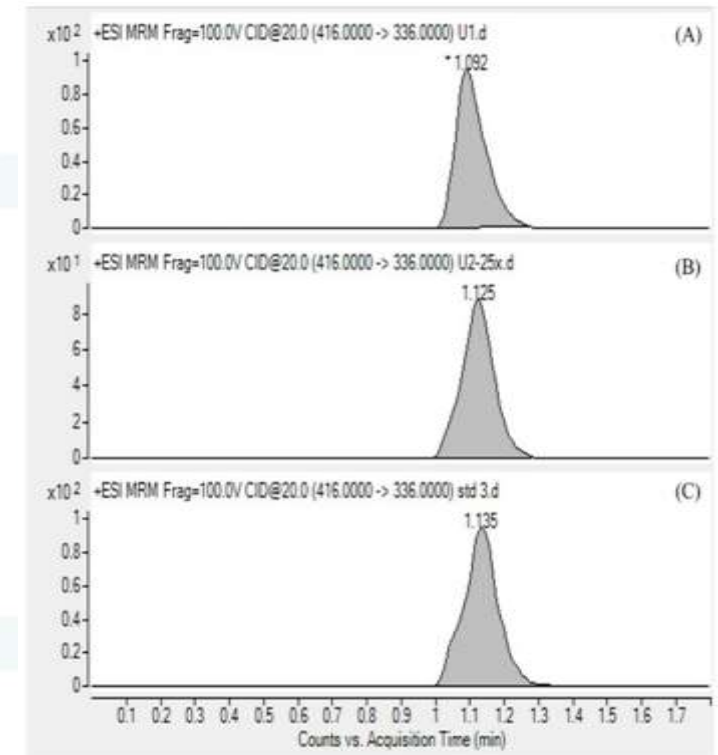


Fig. 5. MRM chromatograms of the *P. nigroviride* biomass samples and analytical standard CYN. A) Chromatogram of the sample from biomass extracted at the 21. day of cultivation; B) Chromatogram of the biomass sample from the late stationary growth phase; C) Chromatogram of the analytical CYN standard.

Davidović et al., 2025 Harmful algae

5) Environmental study - monitoring of microbiological water quality

- ✓ Cyanobacterial blooms in freshwaters in Serbia
- ✓ Identification of blooming genera
- ✓ Isolation of water blooming cyanobacterial strains



This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 872662

✓ Toxigenic cyanobacteria detection

- ✓ Toxigenic cyanobacteria - potential for cyanotoxin production
- ✓ Very common in surface freshwaters during cyanobacterial harmful blooms (CyanoHABs)
- ✓ From the aspect of risk assessment timely determination of potential toxin-producers is of crucial importance



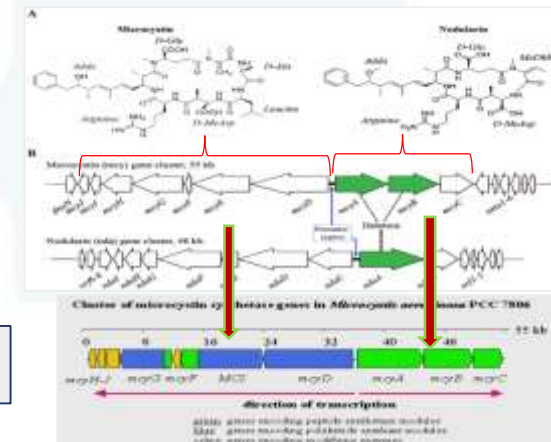
Photos by prof. Z. Svirčev

- The main focus is **the most common toxins in surface waters - hepatotoxins microcystins (MCs)**
- MC synthesis is determined by a multifunctional enzyme complex **MICROCYSTIN SYNTHETASES:**

1. Non-ribosomal peptide synthetase (**NRPS**)
2. Polyketide synthase (**PKS**)

encoded by **mcy genes**

•MCY genes as molecular markers and early-warning predictors of toxin production



- Amplified fragments of MCY gene markers are used for the determination of MC-producers
Usually 1 gene (mcy B or mcy E) is used

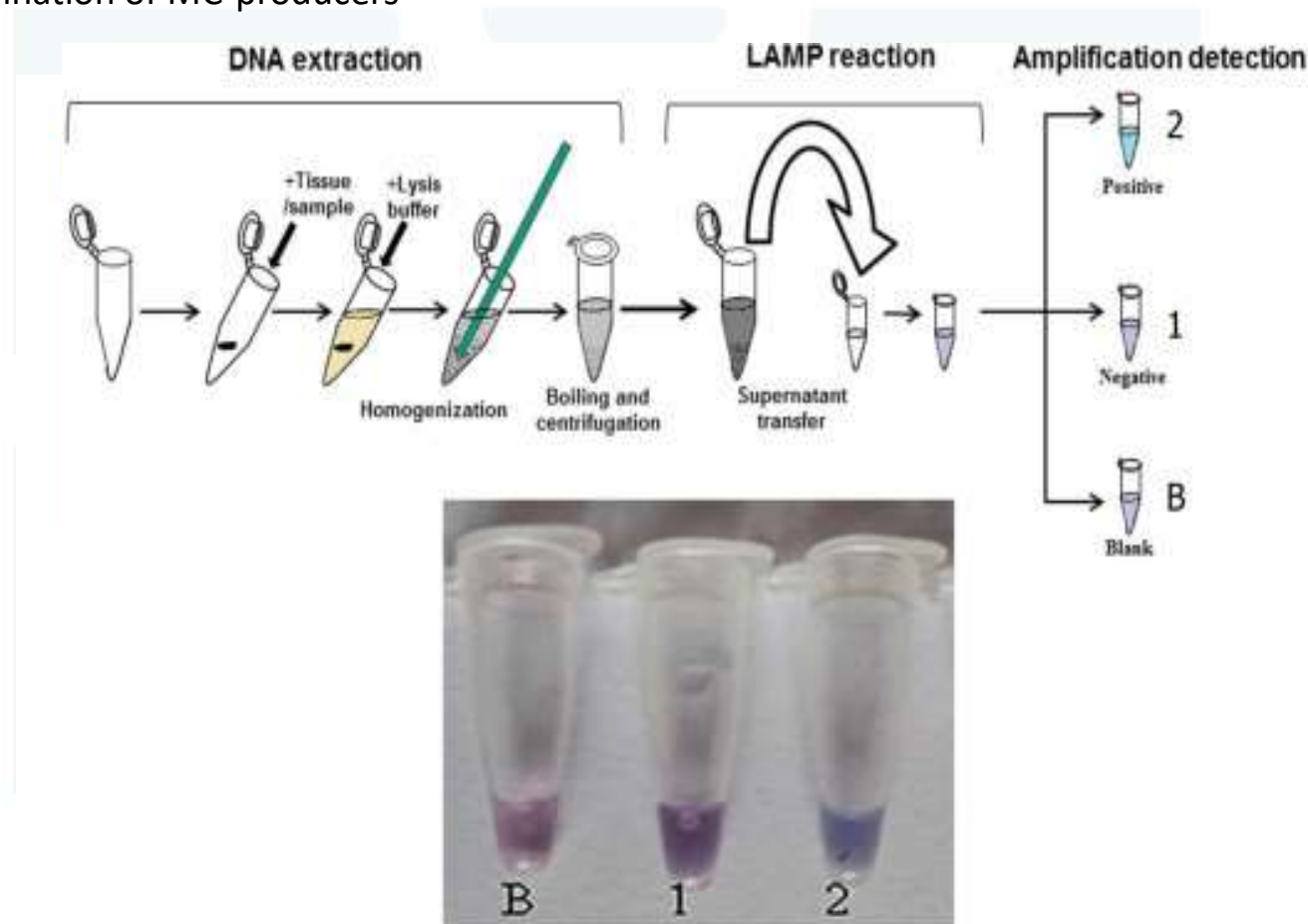
- Genomic DNA is used as the template

1. Isolation and purification of genomic DNA
2. Checking the quality and quantity of DNA
2. **PCR (qPCR) analysis using specific primers**
3. Agarose gel electrophoresis


- **PCR - replacement by LAMP**

(Loop-mediated isothermal amplification)

✓ **simpler, faster and more affordable**



Toxigenic cyanobacteria detection

- ✓ Loop-mediated isothermal amplification (LAMP) method - amplifies the target gene under **isothermal conditions** with an optimum T between 60 to 65°C
 - ✓ uses 4-6 primers recognizing 6-8 distinct regions of target DNA for a **highly specific** amplification reaction
 - ✓ **sensitivity** can be 100 to 1000 fold higher than the PCR
 - ✓ **enhances amplification rate** -10⁹ copies
 - ✓ helps detection of the target in less time –**less than an hour**
 - ✓ amplifies even a **small quantity of the target DNA**- can help in the early detection of the toxigenic cyanobacteria
 - ✓ alternative method -**economical and executable in low-resource laboratory settings**
- 

Final goal

- ✓ the development of LAMP-based diagnostic tools in a paper/strip format or the integration of this method into a microfluidic platform such as a Lab-on-a-chip - it can be a high improvement in the concept of toxigenic cyanobacteria detection



Augustine et al. 2020 Biology

ONGOING PROJECTS

RECAP- Integrated Strategy for Rehabilitation of Disturbed Land Surfaces and Control of Air Pollution (The project is funded by the Science Fund of the Republic of Serbia)



BIONEER- Management of post-mining waste areas through cyanobacterial bioengineering (The project is funded by the KONE Foundation Finland)



COST Action - Effective Lake management: reducing cyanobacteria by actions in the catchment (CYANOACTION) CA23160





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Novi Sad – Downtown



Novi Sad – Petrovaradin Fortress



Krusedol Monastery



in the Fruska Gora National Park

Ravanica Monastery



Staro Hopovo Monastery



Novo Hopovo Monastery





Thank you for your attention!