EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

www.cyanocost.com

CYANOCOST – ES 1105 Action

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

Short Term Scientific Mission (STSM)

Title: Methods in genotoxicity testing of cyanobacteria and their metabolites

Researcher

Michal Bittner, Ph.D.
Faculty of Science
Masaryk University
Brno
Czech Republic

Research Centre for Toxic Compounds in the Environment – RECETOX
Group leader: Prof. Luděk Bláha, Ph.D.

Host Organization

National Institute of Biology - NIB
Department of Genetic Toxicology and Cancer Biology
Ljubljana, Slovenia
Group leader: Prof. Metka Filipič, Ph.D.
Supervision: Bojana Žegura, Ph.D.

Objectives

Objectives of the STSM was to learn basic methods on genotoxicity testing *in vitro using* :

I) Single cell gel electrophoresis, Comet assay (reveals single and double strand breaks, alkali-labile sites, DNA–DNA/DNA–protein cross-links and single strand breaks associated with incomplete excision repair).
II) Micronucleus test (biomarker of effect, relevant for risk assessment of cancer).
III) Practical genotoxicity testing of cyanobacterial samples using upper mentioned methods.

Methodology

I) Comet assay: HepG2 cells were seeded onto12-well plates at a density $8*10^4$ cells/well in 1mL of cultivation media and left overnight at 37 ° C and 5% CO_2. Then, cells were exposed to cyanobacterial extracts. After 24h, cells were harvested and single cell gell elfo was carried out according to Žegura and Filipič, 2008. Finally, agarose slides were stained using ethidium bromide, and evaluated using fluorescent microscope/ image analysis software.
II) Cytokinesis-block micronucleus (CBMN) cytome assay : HepG2 cells were seeded to $25cm^2$ flasks at density $5*10^5$ cells/flask in 5mL of cultivation media and left overnight at 37 ° C and 5% CO_2. After 24 h, cells were exposed to cyanobacterial extracts for 24 h. Then, exposure media was removed, and cells were exposed to cytochalasin B. After 24h, cells were harvested, and the method was performed according to Fenech, 2010. For evaluation, the micronuclei were scored in acridine orange stained slides using fluorescence microscope.
III) Genotoxicity testing of cyanobacterial extracts: Upper described methods were used to test 10 extracts from cyanobacterial biomasses that were prepared at RECETOX before STSM. Samples were tested at levels ranging 0.02 - 2 mg_{DM}/mL. The samples were coded.

Results

Genotoxic effect in the mean of DNA strand-breaks (Comet assay) was shown for samples 1, 3, 6 and 9 (Fig. 1). Genotoxicity testing using micronucleus test haven't been evaluated yet.

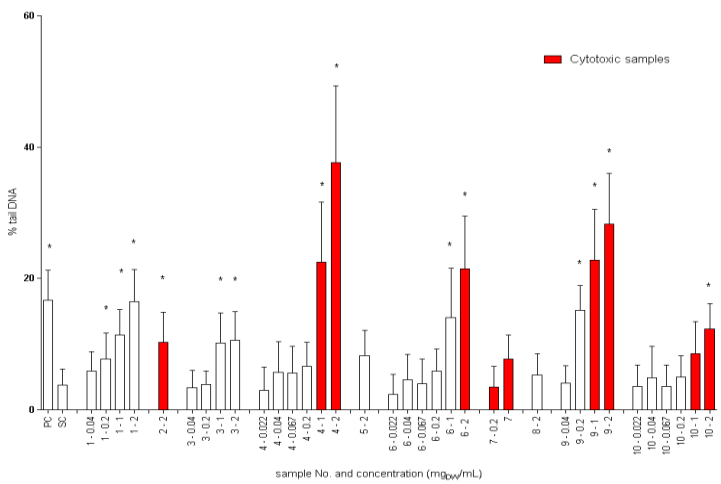

Fig. 1. Results of Comet assay shown as percentage of tail DNA. PC – positive control B[a]P, SC – solvent control MeOH. Red columns represent samples that were significantly cytotoxic. Values represent mean+SD of two independent assessments. Asterisk denotes values different ($p < 0.05$) from SC.

Highlights

- Various cyanobacterial extracts were successfully assessed for genotoxicity using Comet assay and Micronucleus test.
- Four out of ten samples were positive in Comet assay at non-cytotoxic levels.
- New collaboration between NIB and RECETOX was established.

References

- Fenech, M. 2000." *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis* 455:81–95.
- Žegura, B. and M. Filipič. 2008. P. 418 in *Optimization in Drug Discovery*, Totowa, NJ: Humana Press Inc.

COST is supported by the EU RTD Framework Programme

ESF provides the COST Office through a European Commission contract