Toxicity of *Raphidiopsis raciborskii* extracts

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INTRODUCTION

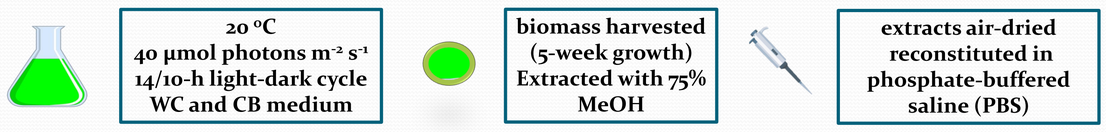
Raphidiopsis raciborskii (Wołoszyńska) Aguilera & al. 2018 is a cosmopolitan cyanobacterial species known for its ability to produce numerous bioactive secondary metabolites. The diversity of its biological activity has sparked significant interest in exploring the harmful potential of strains from various geographical origins.

MATERIAL AND METHODS

Cyanobacterial cultures

R. raciborskii strains from different geographical origin and toxicity were used:

- Six non-toxic strains isolated from the lakes in Poland (UAM/DH-KmRr, UAM/DH-MRr, UAM/DH-BiRr, UAM/DH-PnRr, UAM/DH-BoRr, UAM/DH-ZmRr)
- One non-toxic strain from Lake Kinneret, Israel (KLL07)
- Two *R. raciborskii* CS-505 and CS-506, cylindrospermopsin (CYN) producers, isolated from Solomon Dam, Palm Island, Queensland - obtained from the Australian National Algae Culture Collection, CSIRO
- *Microcystis aeruginosa* PCC 7813 from the Pasteur Culture Collection of Cyanobacteria (PCC), microcystin producer used as a positive control in some assays



ACUTE TOXICITY ASSAY *Artemia salina*

Commercial mixture of *A. salina* eggs and salts were incubated for 30-36 hours under controlled conditions, at a temperature of 30°C with constant illumination and aeration. After hatching, juvenile individuals aged up to 24 hours were selected for use in the experiment. Each extract was tested at 3 concentrations. The obtained values were used to express the mortality/survival rate as a percentage.

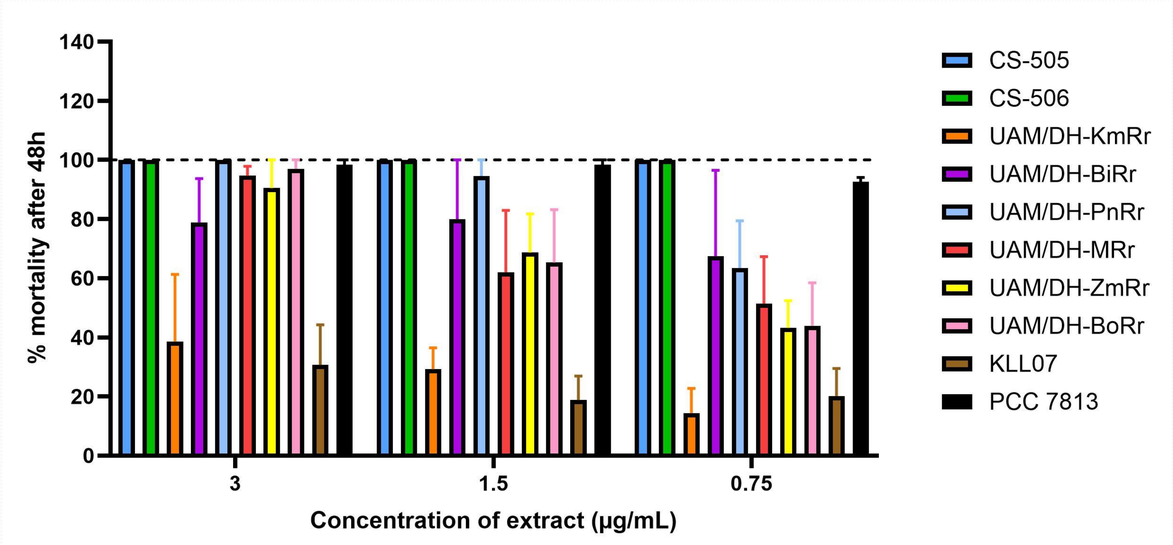


Figure 1. The percent of mortality (%) of *A. salina* larvae after 48h of exposure to cyanobacterial extracts in varying concentrations.

Australian strains (CS-505, CS-506) and the *Microcystis* strain PCC 7813 are highly toxic to *A. salina* larvae. In contrast, several *R. raciborskii* strains, especially KLL07 and UAM/DH-KmRr, show lower or negligible toxicity.

MTT cell viability assay

The MTT test is a colorimetric assay based on measuring the conversion of yellow thiazolyl blue tetrazolium bromide (MTT) into purple-blue formazan crystals, due to the action of mitochondrial dehydrogenase in metabolically active, viable cells (human neuroblastoma cell line SH-SY5Y). The results are expressed as: % survival=100 - % of mitochondrial dehydrogenase, where % of mitochondrial dehydrogenase= absorbance(sample)*100/ absorbance(control)

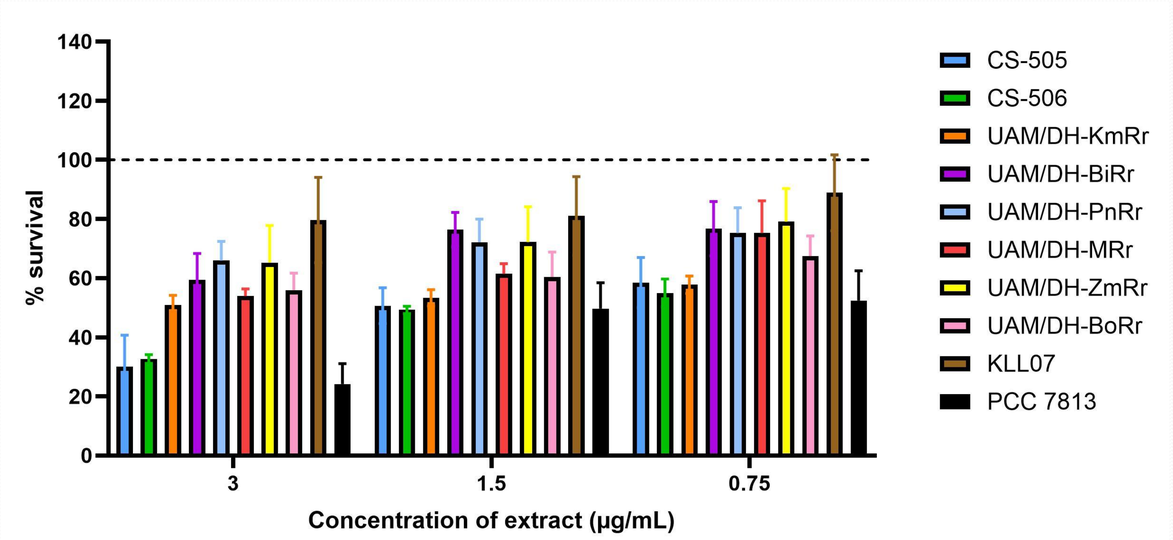


Figure 2. The percent of cell survival (%) after 48h of exposure to cyanobacterial extracts in varying concentrations.

PCC 7813 (*Microcystis* strain) is clearly the most cytotoxic, aligning with known production of potent bioactive compounds. CS-505 and CS-506 (*R. raciborskii*, Australian strains) show moderate toxicity. In contrast, several strains, notably KLL07, are consistently non-toxic or weakly toxic.

GAP JUNCTIONAL INTERCELLULAR COMMUNICATION (GJIC)

Gap junctional intercellular communication (GJIC) was evaluated using the rat liver epithelial cell line WB-F344, a normal, non-tumorigenic line that exhibits properties of hepatic progenitor or so-called oval cells. GJIC was assessed using a multiparametric scrape-loading dye transfer assay (mSLDT), conducted simultaneously with cytotoxicity endpoints (cell density and the percentage of dead cells). The effects of methanolic extracts were evaluated after both short-term (30 min) and prolonged (24 h) exposure, in order to detect both rapid-potentially transient-inhibition of GJIC as well as GJIC inhibition caused via slower mechanisms.

Cyanobacterial extract	GJIC		Total Cell Density		% Dead cells	
	EC50		**EC50**		**EC10**	
	30 min	24 h	30 min	24 h	30 min	24 h
CS-505	> 1.5	0.42	> 3	2.67	1.76	2.59
CS-506	1.51	0.76	> 3	2.73	> 3	2.71
KLL07	> 3	> 3	> 3	> 3	> 3	> 3
UAM/DH-KmRr	1.36	> 3	> 3	2.02	2.99	2.55
UAM/DH-PnRr	1.05	0.18	> 3	2.28	1.51	1.51

Table 1. IC_{50} values (for GJIC inhibition and cell density reduction) and EC_{10} values (for increased cell death) derived using non-linear regression from the dose–response curves.

Both CYN-producing strains (CS-505 and CS-506) almost completely inhibited GJIC, with CS-506 showing rapid inhibition at 30 minutes, and stronger effects observed at 24 hours. Both extracts inhibited GJIC at concentrations below 3 mg d.w./mL, which was cytotoxic. The CYN-non-producing strain KLL07 showed no significant cytotoxicity and only partial GJIC inhibition at 24 hours. The Polish CYN-non-producing strains, UAM/DH-KmRr and UAM/DH-PnRr, inhibited GJIC, but with different time-response profiles: first one induced rapid GJIC inhibition at 30 minutes, which diminished over time, while the second showed increasing inhibition over time. The diverse effects observed in WB-F344 cells strongly suggest the varying presence of bioactive metabolites, distinct from CYN, among different *R. raciborskii* strains. These metabolites can elicit potent cytotoxic and pro-tumorigenic effects and may be found in investigated CYN-non-producing strains isolated from Polish or Israeli lakes.

ALKALINE COMET ASSAY

Human peripheral blood was collected by venipuncture from three healthy donors. The level of DNA damage was assessed using an alkaline version of the single-cell gel electrophoresis/comet assay. To prevent potential photo-induced DNA damage, all experiments were carried out in the dark.

%DNA damage = Class1 + Class2 + Class3 + Class4

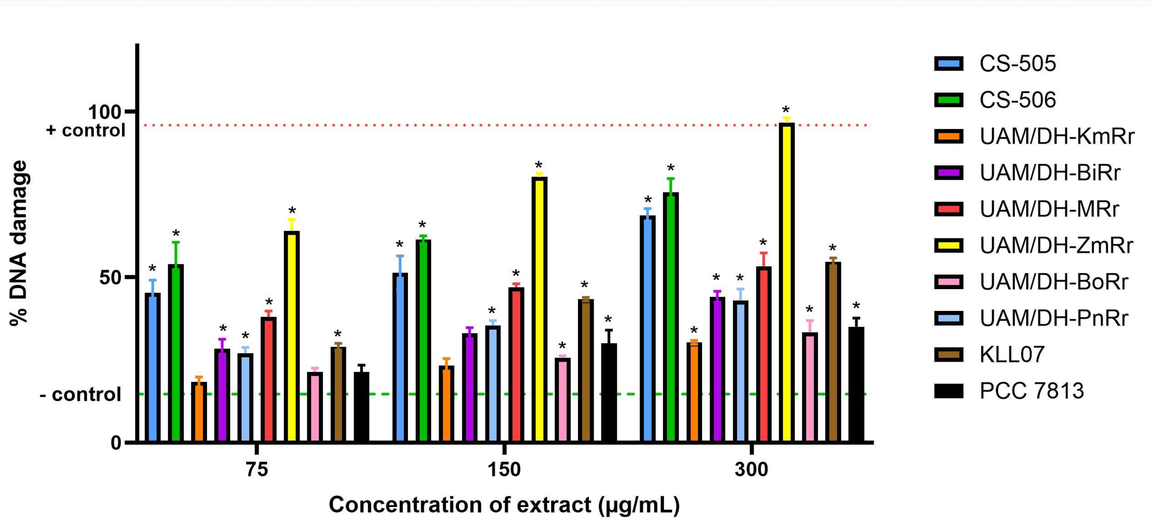


Figure 3. The percent of DNA damage in peripheral blood lymphocytes of healthy persons in treatment with methanolic extract of cyanobacteria biomass in vitro (ANOVA, $p < 0.0005$).

The results of the *in vitro* genotoxic effect of the methanol extracts from different cyanobacteria biomass samples on PBLs exhibited a significant increase of the %DNA damage compared to the negative control. The significantly highest %DNA damage was observed in the treatment with the methanol extract of UAM/DH-ZmRr, in all tested concentrations, while the significantly lowest %DNA damage was detected in the treatment with UAM/DH-KmRr extract compared to the other analyzed extracts. UAM/DH-KmRr < UAM/DH-BoRr < PCC 7813 < UAM/DH-PnRr < UAM/DH-BiRr < KLL07 < UAM/DH-MRr < CS-505 < CS506 < UAM/DH-ZmRr

CONCLUSIONS

Cyanobacterial extracts seem to contain potent bioactive compounds with harmful effects on other organisms, cellular metabolism and GJIC, and genetic stability. Toxic effects included mortality of invertebrates, disruption of cellular metabolism and GJIC, as well as DNA damage of the blood cells. Even though only few strains contain known cyanotoxin, the described effects were found in other strains as well, suggesting a presence of some other biologically active compounds that could potentially explain the observed effects. Strains PCC 7813, CS-505, and CS-506 showed the highest toxicity and genotoxicity, while KLL07 consistently displayed minimal effects. These results suggest that cyanobacterial extract have dose-dependent and strain-specific effect. The described biological activity of *R. raciborskii* highlights potential ecological and health risks associated with this cyanobacterial species.

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