GJIC ASSESSMENT INDICATES NOVEL TOXIC AND CARCINOGENIC THREATS FROM THE INVASIVE WATER BLOOM CYANOBACTERIUM *RAPHIDIOPSIS RACIBORSKII*

Polina Shpet[1,2*], Petra Labohá[1], Nada Tokodi[3,4], Dariusz Dziga[4], Pavel Babica[1,5]

[1] *RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2] *Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Brno, Czech Republic*
[3] *Laboratory of Metabolomics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland*
[4] *Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia*
[5] *Department of Experimental Phycology and Ecotoxicology, Czech Academy of Sciences, Brno, Czech Republic*

** polina.shpet@recetox.muni.cz*

Introduction

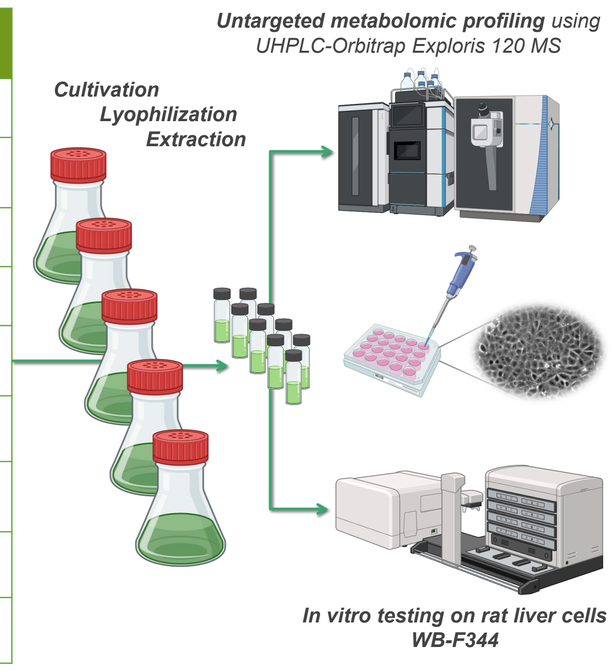
- ***Raphidiopsis raciborskii*** is a bloom-forming cyanobacterium found worldwide. Strains from Oceania and Asia often produce **cylindrospermopsins (CYNs)**, while South American strains may produce **saxitoxins (SXTs)**.
- **European and Mediterranean strains** do not appear to synthesize CYNs or SXTs, but they may generate other **bioactive or toxic metabolites**.
- To explore this, we analyzed ***R. raciborskii* isolates from Polish lakes** by **untargeted metabolomics**, comparing them with a CYN-negative strain from Israel and two CYN-producing strains from Australia.
- Toxicity was assessed *in vitro* using rat liver stem-like cells (WB-F344), focusing on effects on **gap junctional intercellular communication (GJIC)** and **cytotoxicity markers**.
- Since GJIC is crucial for tissue homeostasis and its disruption is linked to **carcinogenesis**, this study evaluated whether *R. raciborskii* metabolites interfere with this process.

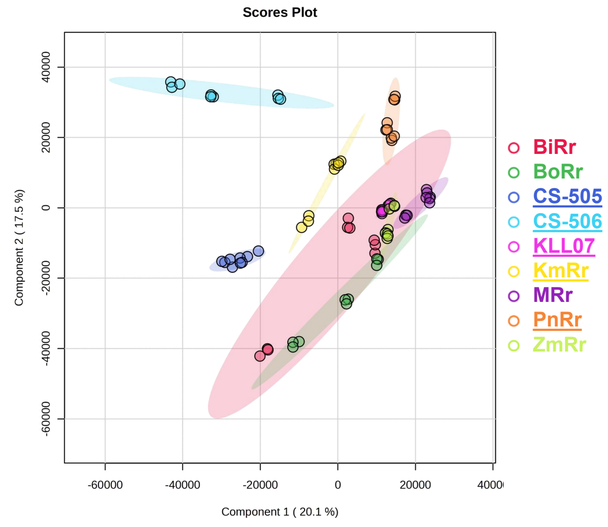
Major Findings

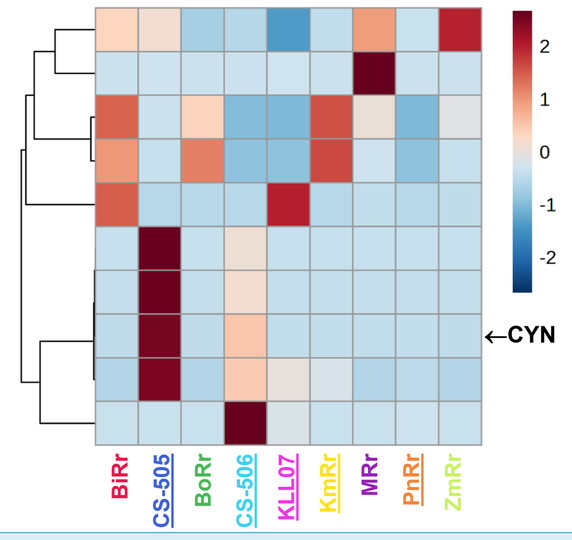
- Metabolomic profiling revealed distinct metabolite patterns among strains **CS-505, CS-506, KLL07,** and **UAM/DH-KmRr** and **-PnRr**.
- These strains showed varying **concentration- and time-dependent inhibition** of **GJIC** and **cell viability** in rat liver cells.
- **Toxicity did not correlate with CYN production.** Notably, the **CYN-lacking strain UAM/DH-PnRr** (from Pniewskie Lake) caused **the strongest GJIC inhibition** and **cytotoxicity**.
- **GJIC inhibition** occurred at **non-cytotoxic extract concentrations**, suggesting interference with receptors or signaling pathways.
- **Inhibition patterns differed by strain**, appearing after short or prolonged exposures, implying distinct mechanisms of action.
- Because **GJIC disruption is a hallmark of nongenotoxic cancer promotion**, these findings indicate that ***R. raciborskii* produces metabolites with potential pro-tumorigenic activities**. GJIC assays may therefore serve as a useful tool for identifying such metabolites.

Strain code*	Origin	CYN
CS-505	Australia	✓
CS-506	Australia	✓
KLL07	Israel	✗
UAM/DH-BiRr	Poland	✗
UAM/DH-BoRr	Poland	✗
UAM/DH-KmRr	Poland	✗
UAM/DH-MRr	Poland	✗
UAM/DH-PnRr	Poland	✗
UAM/DH-ZmRr	Poland	✗

**The underlined strains were selected for the in vitro experiments.*



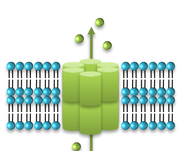




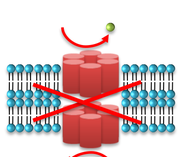
Untargeted metabolomic analysis identified over 880 unique compounds based on their mass-to-charge ratio (m/z) and enabled the annotation of more than 120 compounds. *Left:* **Partial Least Squares Discriminant Analysis** revealed that the CYN-producing strains **CS-505** and **CS-506**, along with two Polish strains (**UAM/DH-KmRr** and **UAM/DH-PnRr**), displayed metabolic profiles most distinct from the other strains, including **KLL07**, which clustered together. *Right:* **Hierarchical clustering** revealed that different strains produced distinct dominant metabolites. Based on these results, the strains **CS-505**, **CS-506**, **KLL07**, **UAM/DH-KmRr**, and **UAM/DH-PnRr** were selected for the evaluation of effects in mammalian cells *in vitro*.

Gap junctional intercellular communication (GJIC)

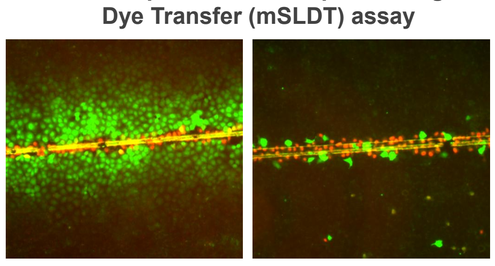
Adjacent cells in healthy mammalian tissues communicate via **gap junctions**, which are membrane channels formed by **connexin** proteins.

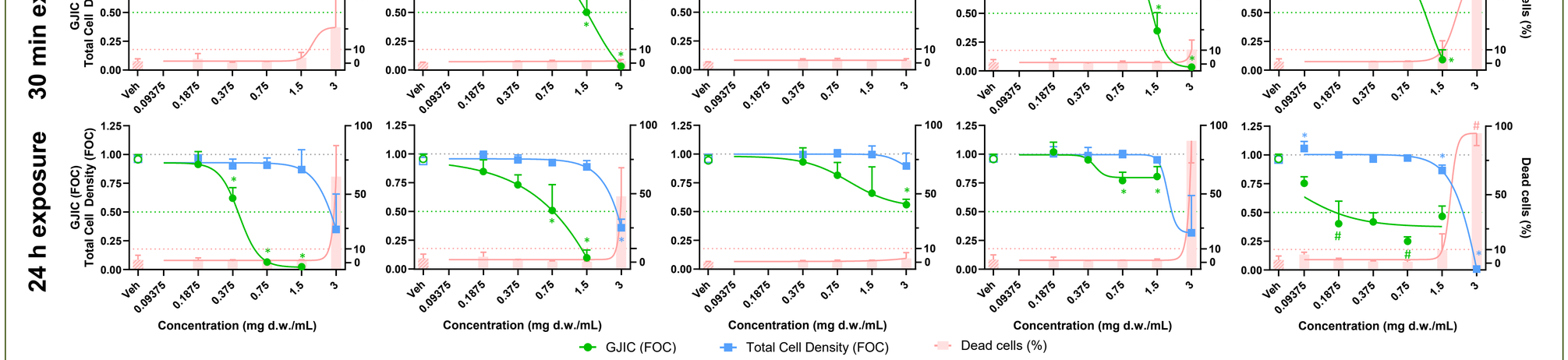


Disruption of GJIC is associated with various **diseases**, including **cancer**, often in response to **chemical exposures**.



In vitro GJIC assessment by a **multiparametric Scrape Loading Dye Transfer (mSLDT) assay**





Effects of the *R. raciborskii* extracts on GJIC, Total Cell Density, and % Dead cells in rat liver cells WB-F344. GJIC and Total Cell Density are presented as a Fraction of the Control (FOC) relative to the untreated control (FOC=1) and plotted on the left y-axis. % Dead cells are expressed as the percentage of dead cells from the total cell count in the culture and plotted on the right y-axis. Data represent means ± S.D. from independently prepared and tested extracts. Treatments significantly different from the vehicle control (Veh) based on ANOVA/Dunnett's test are marked with *, while treatments with non-normal distribution and/or unequal variances were tested by Kruskal-Wallis ANOVA on ranks/Dunn's test with significant differences marked with #. The summary of IC_{50} and EC_{10} values (mg d.w./mL) estimated using non-linear regression is provided in the Table.

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