Novel toxic activity associated with the cyanobacteria *Limnothrix*: use of screening assays for detection

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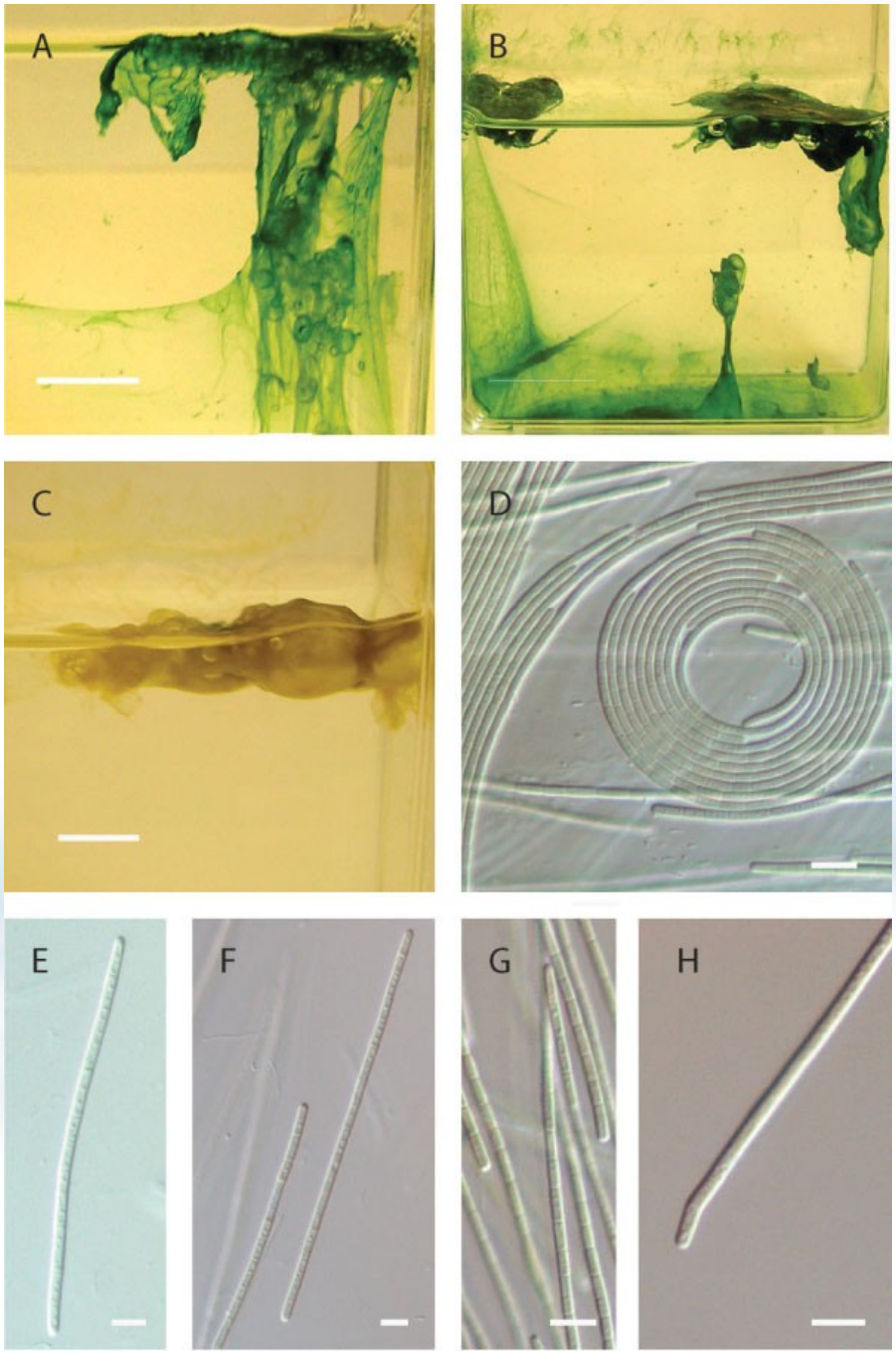
Background

•Larelle Fabbro/Cathy Bernard-Pattinson were looking for unexplained toxicity in isolates from central QLD

• Exploring possibility that cylindrospermopsin (CYN) occurred in unidentified spp.

• *in vitro* toxicity screening assays were used at AWQC to look for CYN by instead detected novel toxic activity.



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- gross morphology of the benthic and surface accumulations

- Colour plates from Bernard *et al* (2010) Environmental Toxicology DOI 10.1002/tox

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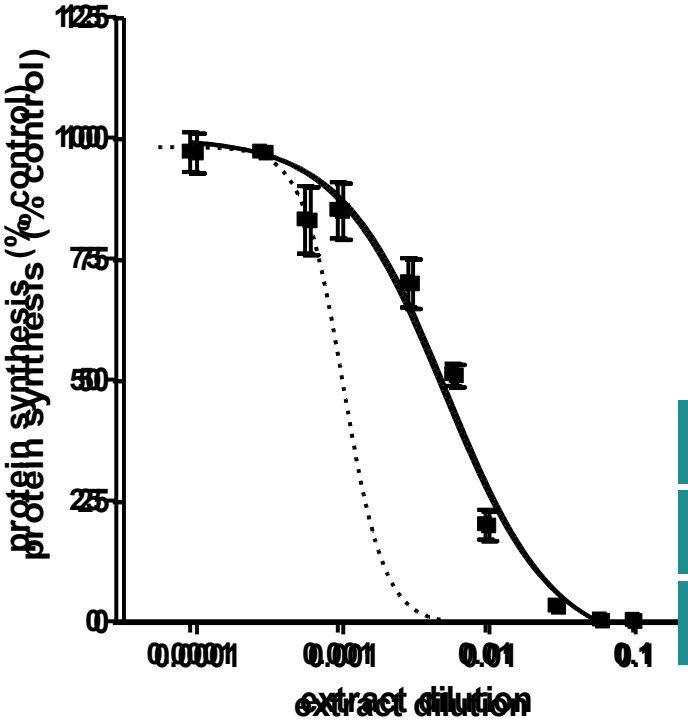
Screening of *Limnothrix* extract for toxic activity

- Extracts were screened for CYN using an *in vitro* protein synthesis inhibition assay
  - diagnostic for CYN (mode of action)

- Preliminary data showed extract was positive in assay – hypothesized CYN present.

- However assay results were not backed up by analytical results (HPLC) leading to further investigation



- Extract inhibited cell-free PS in a concentration dependent manner
- Curve does not fit pattern for CYN

CYN not detected by ELISA

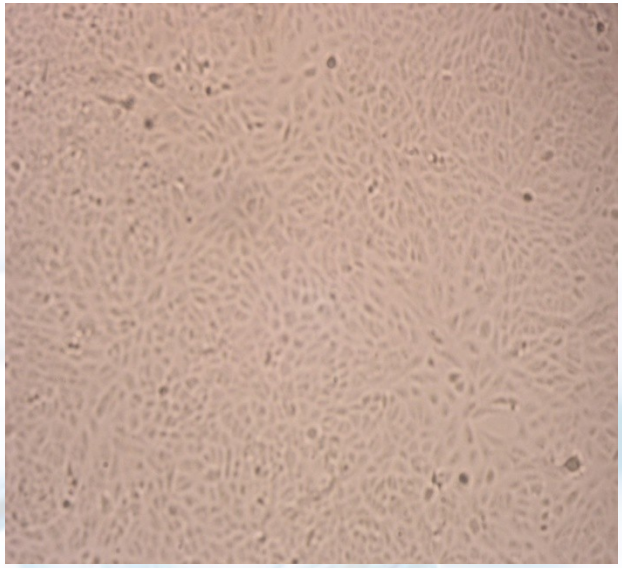
CYN not detected by HPLC & LC/MS

Toxin genes not detected by PCR

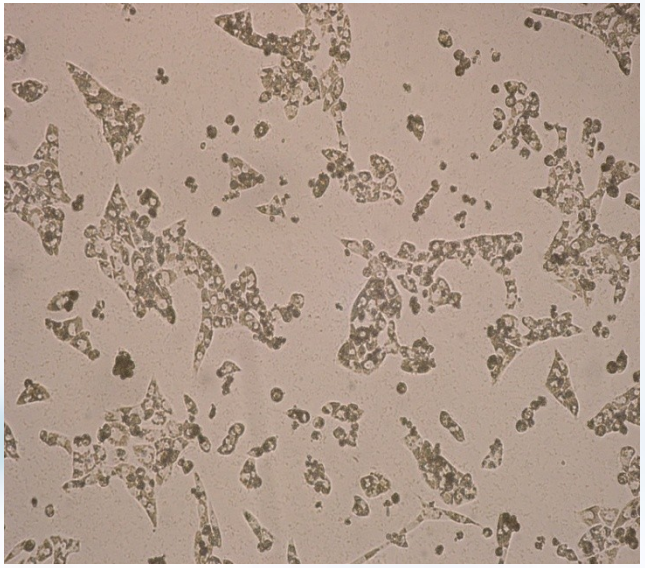
Inhibition of cell-free protein synthesis by AC0243 extract. Data are mean ± SE of three independent experiments. Data were modeled to a sigmoid dose-response curve, r2 = 0.98, slope = 1.2. For comparison, the dashed line indicates a typical concentration response curve for cylindrospermopsin (0.1–100 nM) slope = 2.9. Bernard *et al* (2010). *Environ Toxicol*

Cytotoxicity

control

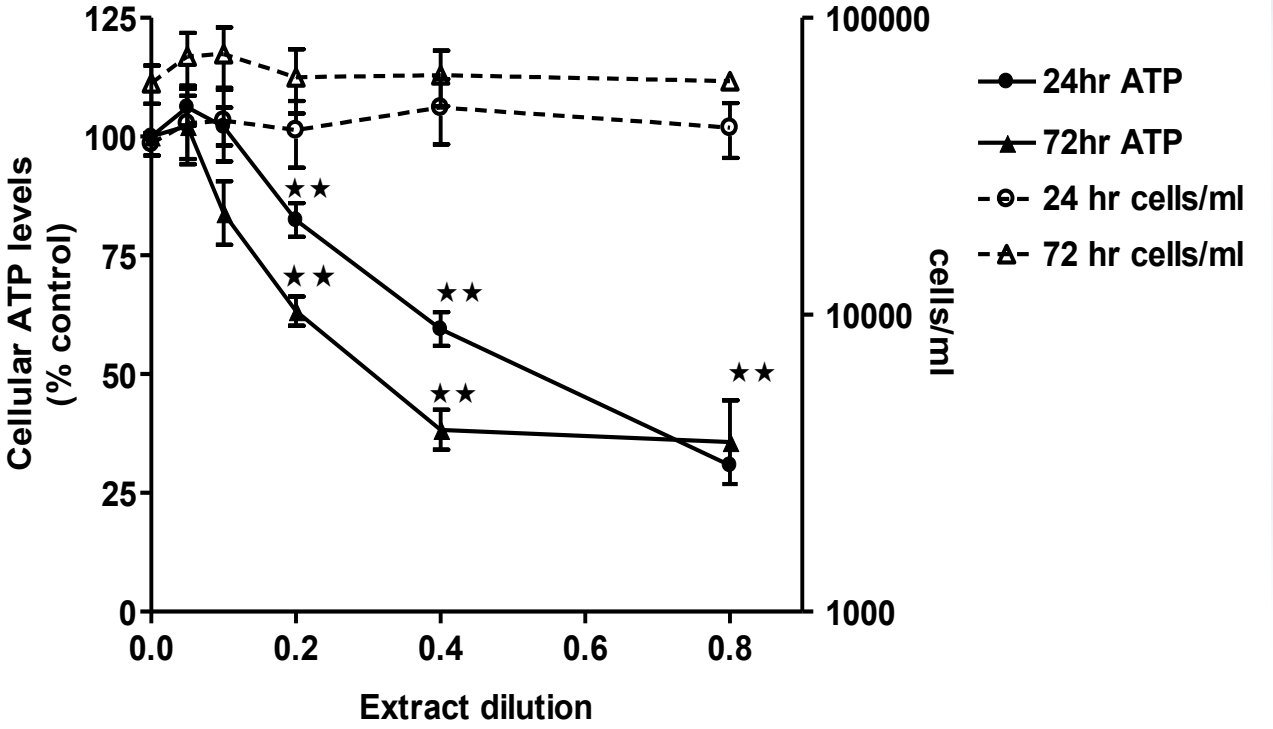


extract



Vero (kidney) cells were exposed to *Limnothrix* extract (8 mg/ml) for 72hr.

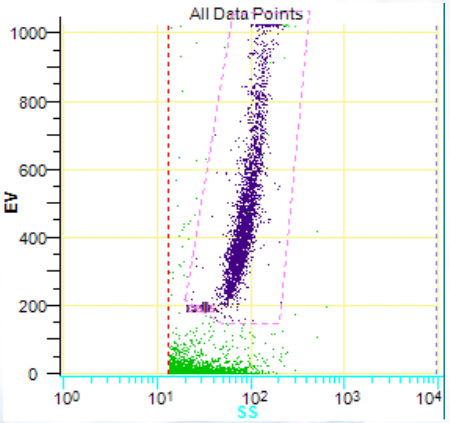
ATP depletion prior to cell death

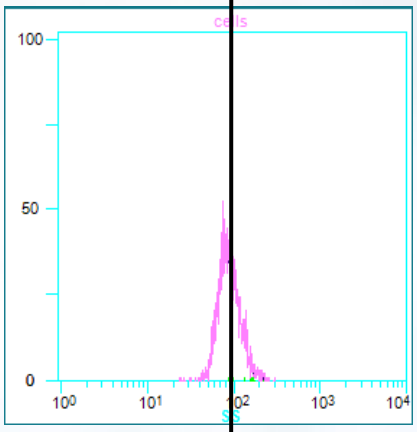


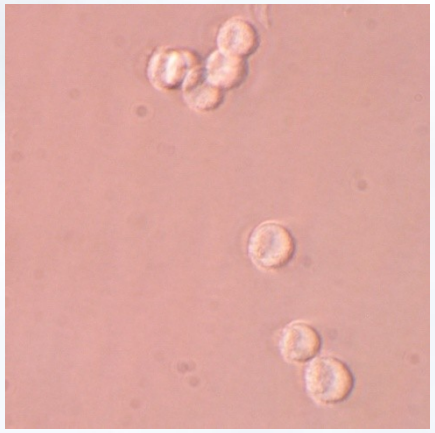
Reduction of cellular ATP levels following treatment of Vero cells with *Limnothrix* extract for 24 hr or 72 hr. Data are mean ± SE of 3 independent experiments. Data were analysed by one-way ANOVA followed by Dunnett's test. Significant differences from the control at each time point is indicated, ** p <0.01. Bernard *et al* (2010). *Environ Toxicol*

Flow cytometry analysis

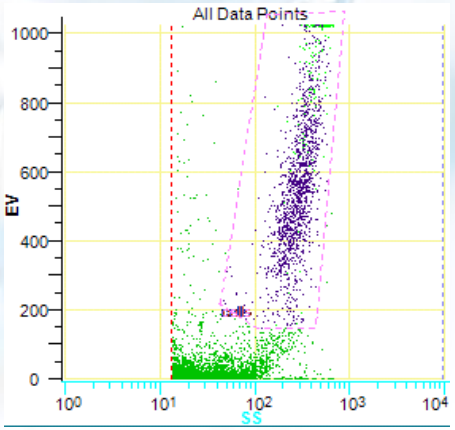
Control
Vero cells

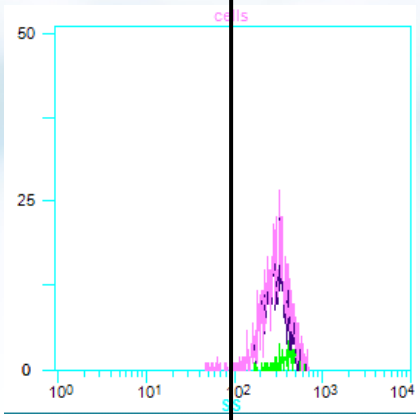


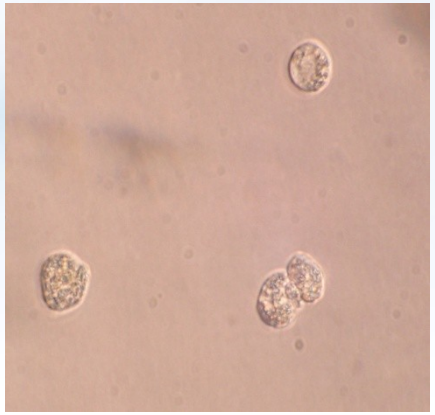




Limnothrix
treated
24 hr







Current status

• *Limnothrix* material is toxic & effects are novel (confirmed in mouse bioassay)

• water soluble toxin - aqueous extracts used

• currently generating data to assess relevance to the water industry

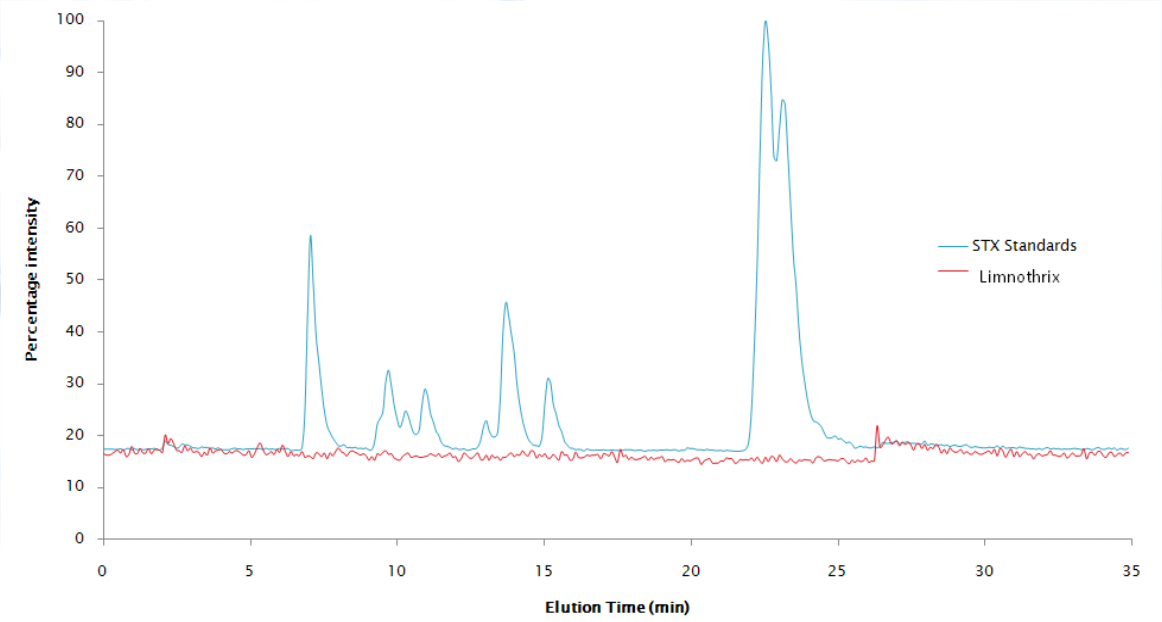
Current work

- Isolation and characterisation of the *Limnothrix* toxin
  - Current honours project, chemistry student
  - Expect to be continued as PhD project

- Aim to produce method of purifying toxin from extract
  - analytical method for toxin detection
  - provide material for continuing cell culture work
  - does pure compound explain the effects of the extract?

Current work

- Fractionation of *Limnothrix* extract – SPE separation
  - Use of protein synthesis screening assay to determine activity of fractions
  - LC/MS characterisation



Chromatogram obtained from Paul Whan, Honours student AWQC & Adelaide University

Application of toxicity screening assays

- Use of screening assays in the CyanoSurvey project
  - Which species are toxic?
  - Emergence of new toxigenic species, changes in geographical distribution of toxic species
  - Detection of new toxic activity

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Application of toxicity screening assays

- ELISA's for known toxins (CYN, MCYST, STX)
- Protein synthesis inhibition assay (CYN)
- Protein phosphatase inhibition assay (MCYST, nodularin)
- AChE assay (anatoxin-a(s))
- Cell culture assays
  - Neuroblastoma assay (STX)
  - Cell based protein synthesis assay (CYN)
  - Cytotoxicity screening –novel toxicity