Identification, detection & characterisation of cyanobacteria using traditional & DNA-based methods

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Background

- Cyanobacterial blooms are a significant public health issue, particularly in summer.
- Cyanobacteria (blue-green algae) produce a variety of toxins such as hepatotoxins, neurotoxins and dermatoxins which are detrimental to human health.
- As a result, dams are continually monitored for the presence of toxic cyanobacteria and their cyanotoxins.
- The staff in the Phycology Unit at FSS identify and enumerate blue-green algae using phase contrast microscopy.
- LC-MS and HPLC are used at FSS to both identify and quantify cyanotoxins in water samples.
- Our aim at FSS is to provide analytical data to assist in management of potentially toxic cyanobacteria and algal blooms.
Cylindrospermopsin (CYN)-producers

*Cylindrospermopsis raciborskii*

*Aphanizomenon ovalisporum*
Saxitoxin (STX) producers

Anabaena circinalis
Microcystin (MCY)-producers

Microcystis aeruginosa
Nodularin (NOD) producers

*Nodularia spumigena*

Photo: Glenn McGregor
Phycology Unit

Services & activities:

• Total phytoplankton counts
• Chlorophyll-a quantitation
• Cell biovolume measurements
• Participation in quality assurance with other phytoplankton enumeration labs
• Consultancy
• NATA accredited
• Provision of cultures for research purposes
Phycology Culture Facility
Why use DNA-based methods to study cyanobacteria?

• Identification of cyanobacteria by microscopy:
  - requires extensive specialised training, labour-intensive
  - can be difficult for some species (eg *A. circinalis*, *M. aeruginosa*)
  - cannot differentiate between toxic and non-toxic strains of the same species

• Toxin analysis:
  - expensive and time-consuming
  - effective only when toxin is present
What is PCR? (Polymerase Chain Reaction)

- PCR is a biochemical method of making millions of copies of a gene of interest (e.g., one involved in toxin production).
- Allows detection of very small amounts of the gene of interest.
PCR for a gene of interest

Agarose gel detection

PCR
PCR assay for the presence of pks gene for CYN production

Marker    C.raciborski    Environmental samples    A.ovalisporum    A.circinalis    No DNA Control

Original assay per Schembri et al. 2001
Detection of cyanobacteria using PCR

- At FSS we use PCR assays for detecting CYN, SXT, MCY- and NOD-producing species in treated & untreated dam water
- Agarose-gel and real time PCR assays are used
- We are multiplexing toxin assays to enable detection of different toxin-producing species simultaneously
- We are developing quantitative real time PCR to determine how many copies of a toxin gene may be present in a sample
- Methods are still in development – not yet offered as a routine service
What else are we using PCR for?

**Characterisation of different strains of N.spumigena**

- In 2008, two recreational lakes in SE QLD had blooms of toxic *N.spumigena*
- First report of this organism in Queensland
- PCR was to characterise three different genes from the 8 isolates we established from the blooms
- DNA sequencing data was compared with that from isolates collected in the 1990s from temperate regions of Australia
- The “tropical” strains isolated from QLD were almost identical to each other and to the “temperate” strains
What else are we using PCR for?

• Speciation of cyanobacteria (using 16S rRNA sequencing) that may be difficult to speciate by traditional taxonomy (e.g., picoplankton)

• DNA-fingerprinting of strains (genotypes) within C. raciborskii to see whether there are temporal and/or geographic differences between strains

• To determine the relative ratios of toxic and non-toxic genotypes of C. raciborskii in a single water reservoir over time
Significance of DNA-based methodology to Public Health

- Detection and quantitation of cyanobacteria that *potentially* produce the toxins CYN, STX, MCY, NOD
- Understanding how populations of *C.raciborskii* change in response to environmental parameters may assist with the future management of this toxic species
The FSS Team

• **Phycology Unit**
  - Karen Reardon, Lindsay Hunt, Toni Menjivar, Priya Muhid

• **Toxin analysis**
  - Geoff Eaglesham & Steve Carter

• **Nodularia project**
  - Ian Stewart & Glenn Graham (FSS), Glenn McGregor (DERM), Wasa Wickramasinghe (Entox, UQ), Ross Sadler & Glen Shaw (Griffith Uni)