A new potentially toxic cyanobacterium from Australian freshwaters: First report of the cyanotoxins cylindrospermopsin and deoxy-cylindrospermopsin from *Raphidiopsis mediterranea* (Cyanobacteria/Nostocales)

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Knowledge of candidate toxigenic cyanobacterial species is necessary for assessing risks to human and ecosystem health. Continues to be new records of potentially toxic cyanobacteria (including planktonic and benthic species) in the literature. Need for ongoing screening of new strains and species for potential toxicity. This screening is often opportunistic and relies on bloom samples which are generally concentrated and rapidly growing.
Background – *Raphidiopsis*

- Nostocales – however it lacks heterocysts (N-fixing cells)
- Widely distributed genus, although generally considered more common in the tropics
  - Europe
  - Africa
  - Asia
  - North and South America
  - New Zealand
  - Australia
Background – *Raphidiopsis*

- Genus *Raphidiopsis* has been of interest for some years
- Morphologically similar to *Cylindrospermopsis raciborskii*
- Three species from the genus are known toxin producers from other countries
- Known to occur in the plankton of lakes and reservoirs throughout Australia
Background – *Raphidiopsis* toxicity

- *R. curvata* – CYN, deoxy-CYN (China)
- *R. mediterranea* – homoanatoxin-a, anatoxin-a (Japan)
- *R. mediterranea* – “neurotoxic effects in mice” (Egypt)
- *R. brookii* – paralytic shellfish toxins (Brazil)
Queensland records for *R. mediterranea*

- Recorded from 20 reservoirs
- Maximum concentrations ca. $4 \times 10^6$ cells mL$^{-1}$
- Commonly occur throughout the summer months corresponding to lake stratification
Study Site - Lake Clarendon

Location: 80 km south east of Brisbane, SE Qld
Catchment Area: 3.4 km²
Lake Surface Area: 339 ha when full
Full Supply Capacity: 24,276 ML
Current Capacity: 4,294 ML (17.7% full) at 18/07/2010
Methods

- Strain isolation and purification
- Identification and phylogeny
  - Microscopy – morphology and cell measurements
  - 16s rRNA gene
  - nitrogenase reductase gene ($nifH$)
- Toxicology
  - Screening of CYN, anatoxin-a, microcystins, PSTs by HPLC MS/MS
  - genes associated with cyanotoxin biosynthesis ($pks$, $mcyE$, $sxt1$)
**Raphidiopsis mediterranea** (strain FSS1-150/1)

Vegetative cells:
2.3 – 3.8 × 8.7 – 19.8 (– 22.6) µm

Akinetes:
2.9 – 5.8 × 7.8 – 18.8 µm

*C. raciborskii*

*a* = akinete, scale bar 10 µm
Phylogenetic tree showing neighbour-joining analysis of partial 16S rRNA sequences of 14 cyanobacterial strains. (+ denotes CYN producer)
Toxicity – HPLC MS/MS

- Positive for CYN and deoxy-CYN
- Negative for anatoxin-a, microcystins and PSTs
Comparison of CYN and deoxy-CYN concentrations (µg g⁻¹ dry weight) in selected cyanobacteria

<table>
<thead>
<tr>
<th>Species</th>
<th>CYN</th>
<th>deoxy-CYN</th>
<th>Reference</th>
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<tr>
<td>Anabaena lapponica</td>
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<td>Spoof et al 2006</td>
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<td>Aphanizomenon ovalisporum</td>
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<td>Shaw et al 1999</td>
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<td>Aphanizomenon flos-aquae</td>
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<td>Preußel et al 2006</td>
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<td></td>
<td>1020</td>
<td>102</td>
<td>Li et al 2001a</td>
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<td></td>
<td>6600</td>
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<td>Saker &amp; Eaglesham 1999</td>
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<td></td>
<td>2000</td>
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<td>Raphidiopsis curvata</td>
<td>56</td>
<td>1300</td>
<td>Li et al 2001b</td>
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<td>Raphidiopsis mediterranea</td>
<td>917</td>
<td>1065</td>
<td>This study</td>
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<td>Lyngbya wollei</td>
<td>0 – 33</td>
<td>0.5 – 546.8</td>
<td>Seifert et al 2007</td>
</tr>
</tbody>
</table>
Toxicity – PCR

- Absence of gene clusters associated with cyanotoxin production except \( pks \) (CYN)
- Negative for \( mcyE, sxt1 \)
Other genes – N-fixation (nifH)

- No nifH sequence detected in *R. mediterranea*, in contrast to N-fixing control species (*A. circinalis, C. raciborskii*) – confirming lack of N-fixation machinery
- Consistent with observations of *R. mediterranea* not being able to grow in N-deficient media
Conclusions

- Confirms *R. mediterranea* as a CYN and deoxy-CYN producing species
- Adds to our knowledge of CYN producing cyanobacteria from Australian freshwaters (now 4 species - 3 planktonic, 1 benthic)
- Highlights the need for ongoing and systematic screening of cyanobacterial species and strains
- Demonstrates the utility of molecular methods in discriminating between morphologically similar species/strains and identifying toxin biosynthesis gene sequences

Acknowledgements

- DERM and QHealth for supporting this research
- Steve Carter, Queensland Health Forensic and Scientific Services for chemical analysis of microcystins and PSTs
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- Rebecca Campbell for providing a culture of A. lapponica 996 and for sharing the 16S rRNA partial DNA sequence of this isolate