Case studies: Application of DNA-based tools for cyanobacterial monitoring

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Tim Otten, PhD, MPH
Bend Genetics, LLC
87 Scripps Dr Ste 108
Sacramento, CA 95825
ottentim@bendgenetics.com
www.bendgenetics.com
Presentation overview

Brief overview of cyanobacterial harmful algal blooms (CHABs) and their ecological and human health effects

Principle of real-time quantitative polymerase chain reaction

Examples of QPCR as part of a tiered monitoring framework

Sample collection procedures and the Pros & Cons of QPCR
CyanoHABs are an increasingly common occurrence in many freshwater systems.
Benthic & periphytic CyanoHABs

Benthic *Anabaena* sp. – Eel River, CA

Benthic *Phormidium* sp. – New Zealand

Bouma-Gregson et al., 2017. Harmful Algae 66:79-87

Different CyanoHAB taxa present different cyanotoxin risks
Potential toxins produced by common cyanobacterial genera

<table>
<thead>
<tr>
<th>Cyanobacterial Genera</th>
<th>Anatoxin-a</th>
<th>Cylindrospermopsin</th>
<th>Microcystin</th>
<th>Nodularin</th>
<th>Saxitoxin</th>
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<tbody>
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<td>Aphanizomenon</td>
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<td>Cylindrospermopsis</td>
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<td>Lyngbya</td>
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<td>Raphidiopsis</td>
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Toxicity is a strain-specific trait
- Only cells with toxin genes can produce toxin
- Cells with toxin genes tend to use them (i.e., expression stays turned on)
- QPCR can be used to quantify cyanotoxin gene concentrations
- Because the majority of toxin occurs intracellularly, gene abundance correlates well with cyanotoxin concentration

QPCR “peers” into a cell’s genome
Overview of PCR-based tools

- **Polymerase Chain Reaction (PCR)** – the amplification of specific DNA sequences using complementary synthetic DNA molecules (primers)
  - Sequence information is required in order to design assays
  - Assays can be designed to be strain-specific or universal

- **Real-Time Quantitative PCR (QPCR)** – same concept as regular PCR, but includes a fluorescent dye or probe allowing for **absolute quantification** of gene copies
  - Assumes gene copies/mL equivalent to cells/mL for single copy genes targeted
QPCR as part of a tiered monitoring approach

Is the water visibly green or is a scum present?

- No.
  - Sample bi-weekly
- Yes.
  - Issue advisory
    - OR Conduct cell counts
      - Potentially toxigenic cyanobacteria exceed 4,000 cells/mL?
        - No.
          - Sample weekly
        - Yes.
          - Issue advisory
            - OR Conduct toxin analysis
              - Genera relevant toxins exceed: 20 ppb ATX, 4 ppb CYN, 6 ppb MC, or 10 ppb STX?
                - No.
                  - Sample weekly
                - Yes.
                  - Issue advisory
    - OR Perform Quantitative PCR
      - Do any toxin genes exceed 4,000 copies/mL?
        - Yes.
          - Issue advisory
        - No.
          - Sample weekly
Use of QPCR to assess the toxicity and distribution of Klamath River *Microcystis* sp. blooms
Comparison of methods - Microcystins vs QPCR \( (mcyE) \) estimates


All samples were 0.5 m grab samples
Comparison of methods - *Microcystis* cell counts vs QPCR estimates

Klamath River (2016)

The half-life of DNA in surface water is ~12 hours

Otten, *in prep.*
Comparison of methods - *Microcystis* cell counts vs QPCR estimates

Discrepancy between environmental counts and QPCR estimates not likely explained by (i.e., genome copy number)

Comparison of methods - Microcystins vs QPCR (mcyE) estimates

Klamath River (2016)

Otten, in prep.
Sample collection & archival

- Collect water sample and concentrate by vacuum filtration
  - Filter type is not critical, glass fiber or membrane filters work
  - Larger pore sizes (e.g., < 1 µm) will selectively retain cyanobacteria and other algae
  - Small pore sizes (e.g., 0.2 µm) retain all bacteria
- Don’t freeze water samples before filtering
- Record volume filtered, required for quantification
- Store filters in microcentrifuge tubes at -20°C
  - Samples can be archived for years
Pros & Cons of QPCR testing

Pros

• Faster than cell counting (2-3 hours from start to finish)
• High throughput (40+ samples per analysis batch)
• High sensitivity and specificity
• DNA signal is amplified → good for early detection
• Genes are better correlates of toxin than cell density
• Cheaper than cell counting or toxin testing
• Amenable to other targets (e.g., fecal bacteria)

Cons

• Not a true substitute for toxin testing → tiered strategy
• Cells must be intact to collect their DNA
• Not useful on finished drinking water
• Requires specialized equipment and training
Thanks for your attention!

Please feel free to contact me with any questions.

Tim Otten, PhD, MPH
Bend Genetics, LLC
T: 916-550-1048
ottentim@bendgenetics.com
www.bendgenetics.com