qPCR Analysis of Cyanobacteria Toxins: How it Works and What Your Results Mean

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Toxins Detected by qPCR Analysis

- 16S – Total cyanobacteria
- Microcystins
- Cylindrospermopsins
- Saxitoxins
16S – Total Cyanobacteria

- Commonly called Blue-Green Algae
- It is a Bacteria that can photosynthesize like a plant
- Cyanobacteria grows like any other plant or organism
- The toxins are typically contained within the cells
  - Once released, toxins can remain stable in the water for weeks
- Toxins can be present even when a visible bloom is not
- Not all Toxins are TOXIC
- Blue-green algae, typically lives on the surface of water
- The scum can cause decreased levels of oxygen and prevent sunlight from penetrating the water column
- Toxic blue-green algae can cause lower reproduction and growth rates in the aquatic wildlife, as well as fatality
Microcystin

- Most commonly occurring and toxic cyanobacteria
- Microcystins are a Hepatoxin
- Exposure can come from dermal, ingestion, or inhalation
- Symptoms include:
  - Skin Rashes
  - Abdominal Pain, nausea, vomiting and diarrhea
  - Headaches
  - Sore throat and dry cough
  - Blistering around mouth
  - Pneumonia
  - Liver Disease – interhepatic hemorrhage or hemorrhagic shock
  - Kidney Failure
  - Heart Failure
  - Neurological effects
- Can be fatal to humans and animals
Cylindrospermopsin

- Hepatoxin
  - Liver and Kidney damage
- Exposure is most commonly oral
- Relatively Stable in the dark
- Survives for up to 8 weeks at:
  - 4 - 50°C
  - pH 4-10
- Toxins remain potent after 15 minutes of boiling
Saxitoxin

- Neurotoxin
- Are a large family of toxins that are known as the Paralytic Shellfish Poisoning (PSP)
- Most common exposure is from consuming contaminated shellfish
- Symptoms include:
  - Numbness
  - Headache
  - Dizziness
  - Nausea
  - Loss of Coordination
  - Floating Sensation
  - Muscle Paralysis or Respiratory Failure
qPCR Analysis

- Sample Collection
- Extraction
- Analysis
Sample Collection

- Samples must be collected in an Amber Glass Bottle
- Stored at 0-4°C immediately after collection
- Samples then have to be extracted within 48 hours of collection
- Once extracted and frozen the hold time is extended
Sample Extraction

- DNA Extraction begins with a filtration step using sterile equipment
Sample Extraction
Sample Analysis

- Samples are combined with the Master Mix and pipetted into the plate wells
- Plate is sealed and loaded into the instrument
Sample Analysis

- **Step 1 - Denaturation**: heat plate to 95°C for 15 seconds

  ![Diagram showing denaturation process](image)
Sample Analysis

- **Step 2 - Annealing:** cool plate to 65°C
Sample Analysis

- **Step 3:** the plate repeats step 1 and 2 40 times

- The 40 cycles create an exponential growth of DNA strands
Sample Analysis
Sample Analysis

https://www.youtube.com/watch?v=fkUDuo42xic
Results-16S

- 16S does not distinguish between toxic and non-toxic.
- Can be a helpful guide for anticipating a bloom and assessing source water.
Results - Microcystins

- Generally you will see Gene Detections prior to microcystin Detections
- During the Ohio EPA paired sampling study they found:
  - 100% of Microcystin detections >1.6 µg/L had mcyE detections
  - 90% of these had detections >5.0 gc/µL
  - 100% of Microcystin detections >5.0 µg/L had mcyE detections >5.0 gc/µL
  - <2% of samples had Micrcystin Detections without mcyE Detections
Results – Saxitoxin and Cylindrospermopsin

- In the Ohio EPA paired study <1% of Saxitoxin detections did not have a paired gene detection
- There were no detections for cylindrospermopsin during the study
Questions?