Early Detection of Cyanobacterial Toxins Using Genetic Methods
[Project #2881]

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OBJECTIVES:
This research sought to develop and test rapid genetic methods to identify toxic cyanobacteria. The key objectives were to (1) conduct a literature review and industry questionnaire examining options for rapid genetic tests; (2) characterize and understand the genes involved in cylindrospermopsin and anatoxin production; (3) adapt conventional PCR assays to real-time PCR; (4) develop rapid field methods for DNA preparation; and (5) develop probes for toxin genes and testing in the field.

BACKGROUND:
Toxic cyanobacteria are typically monitored using two basic measures, cell counts and toxicity, each of which is moderately costly, highly operator dependent, and has a slow turn-around time. Rapid genetic detection of these cyanobacteria has the potential to be both faster and cheaper and provide information that complements these existing approaches. The genes that are responsible for the production of cyanotoxins can be used to design probes that will detect toxic species.

HIGHLIGHTS:
• The genes likely to be involved in cylindrospermopsin production in *C. raciborskii* were characterized and attempts were made to identify and sequence genes likely to be involved in the production of anatoxin-a.
• A simple and rapid method for the preparation of cyanobacteria-containing water samples was devised and tested.
• Conventional PCR assays for cylindrospermopsin-producing cyanobacteria were adapted to real-time PCR and tested successfully in the laboratory and the field.

APPROACH:
A comprehensive literature review and industry questionnaire were used to identify and select a suitable platform technology for rapid genetic identification of toxic cyanobacteria. The genes responsible for production of the cyanotoxins, cylindrospermopsin and anatoxin, were identified and characterized using typical molecular techniques including DNA amplification, cloning, and sequencing. The putative role of the genes was established using bioinformatics. Conventional PCR assays for the putative cylindrospermopsin toxin genes were adapted to real-time PCR by designing a gene probe for the PCR product and then optimizing the reaction. A simple field method for the extraction of cyanobacterial DNA was devised and tested after a review of relevant research. The combination of rapid extraction and real-time PCR was used to identify toxic cyanobacteria in the laboratory and field.

RESULTS/FINDINGS:
Questionnaire
Most respondents did conduct routine sampling for toxic cyanobacteria (74 percent) with the majority using microscopy (90 percent) and a lesser proportion toxin testing (24 percent). Many respondents were interested in genetic tests (53
percent) and would prefer a quantitative result (87 percent).

**Toxin Genes**
The structure and organization of the putative cylindrospermopsin genes closely correlated with the proposed mechanism of biosynthesis, but the origin and incorporation of the uracil moiety in the cylindrospermopsin molecule could not be easily explained. A candidate genes cluster for anatoxin biosynthesis was identified.

**Rapid DNA Extraction**
A rapid DNA extraction method for cyanobacteria using microwave irradiation in the presence of detergent was effective when tested on several toxic cyanobacterial species from culture or the environment.

**Real-time PCR Detection**
The reliable detection limit for real-time PCR detection of cylindrospermopsin-producing cyanobacteria on laboratory and field portable devices was 1,000 cells/mL. The comparison of cell and DNA detection limits did not correlate. The assay was able to detect lower cell densities than what would be predicted from the DNA detection results, suggesting there was more than one copy of the toxin gene per cell. In the laboratory, the assay showed specific and reproducible detection while in the field the spatial and temporal variability of toxic cyanobacteria could be rapidly assessed.

**IMPACT:**
The use of real-time PCR as a monitoring tool for toxic cyanobacteria was demonstrated in the laboratory and field. The technology enabled spatial and temporal information on the distribution of toxic algae to be gathered rapidly with replication. These key advantages are not available when using microscopic analysis and provide an important augmentation to routine microscopy and toxin analysis when more detailed and rapid information about key toxic species is required. Further work that investigates how the quantification by real-time PCR relates to cell counts and the incorporation of this information into safety guidelines will maximize the potential of the technology.

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- United Water International
- Metropolitan Water District of Southern California
- GreenWater Laboratories