

Refinements of the qPCR and RT-qPCR detection assays for detecting microcystin producers:
An early warning system for microcystin production

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Abstract

The frequent occurrence of CHABs is a threat to human and ecological health by their toxin products, particularly Microcystin. Microcystin is the most common cyanotoxin produced, with the major toxin-producing genera during CHABs being *Microcystis* and *Planktothrix*. Previously, we've demonstrated that qPCR and RT-qPCR can be used as an early warning detection system for microcystin production in fresh surface waters. However, to form a more robust detection system, certain PCR methods need to be defined and validated. For example, target nucleic acid concentrations and biomass derived from environmental samples can range from low levels (undetectable) to extremely high levels (up to 10^{10} L^{-1}) and thus can impact the overall assays' RT and PCR reaction steps. To create a more robust qPCR and RT-qPCR-based early warning system, two commonly used quantitative PCR systems (TaqMan qPCR and SYBR Green qPCR) were evaluated for their specificity, sensitivity, efficiency, and accuracy. Three potential inhibitory scenarios for these PCR assays and corresponding relief strategies were evaluated for their effectiveness. The resulting qPCR procedures will be evaluated to monitor the occurrences of microcystin producers during the early bloom stages of HABs. Preliminary results from this study will be presented and a proposed protocol will be discussed.

Keywords: Microcystin Producer, Early warning, qPCR, RT-qPCR, Optimization