Development of hand-held, field deployable array biosensors to distinguish multiple Karenia species of red tide dinoflagellates.

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Abstract
In Gulf of Mexico, blooms of Karenia dinoflagellates cause major economic losses, wildlife mortalities, and impinging human health. Development of what Karenia species are present in a bloom, their possible succession, and implications for bloom toxicity is difficult to address due to lack of methods for rapid discrimination. Molecular methods have been developed based on Nucleic Acid Sequence Based Amplification (NASBA) of RNA that can discriminate between algal species nearly indistinguishable by microscopy. NASBA is an isothermal RNA amplification reaction amenable to field deployment using fresh water reagents and a portable heat block. After characterization of novel Karenia spp. qPCR gene sequences, multiplex NASBA primers were developed and tested, initially on in vitro transcripts from cloned genes, then on cellular RNA. Amplification products were distinguishably via field deployable lateral flow microarrays (LFMs). The LFM is used in a dried state, enabling extended deployment. The LFM results for Karenia species exhibited species-specific hybridization patterns, although some cross-hybridization was apparent. Optimization of LFM hybridization stringency provided improved species-specific hybridization. The sequences of the Karenia NASBA amplicons were also used to design molecular beacons (hair-pin shaped oligonucleotides on either end) for use in a multiplexed detection array capable of being read fluorometrically on a real time PCR machine. The beacons were designed such that up to 6 species could be specifically detected in a single reaction helping to provide a method of analysis for bench testing seeded samples that independent of the LFM. Beacons and LFMs are being used to test seeded seawater samples and RNA purified from natural phytoplankton populations; the results of which are presented. 

Introduction

- Toxins from red tide blooms in the Gulf of Mexico cause human health problems and wildlife mortalities. 
- Karenia brevis has been the focus of most studies, but are other recognized species of Karenia significant? 
- What Karenia species are present during a bloom? Morphological discrimination by highly trained individuals has been the focus of most studies, but are other recognized species of Karenia significant? 
- The development of NASBA required qPCR amplifications were performed using Bioanalyzer RNA samples to identify the presence of Karenia species in seawater. 

Analytical Process

- DNA and/or RNA extractions of those species were conducted followed by amplification, cloning, and sequencing of their rbcL gene (for the large Rubisco subunit). 
- In vitro RNA transcripts of the cloned rbcL gene were made for test templates. 
- Species-specific primers were developed,那时 primer mix was used for multiplex amplification scenarios and to simplify methods in further experimentation. NASBA amplifications were performed using Bioanizer lysis kits. 
- Amplification results were quantified using an Agilent Bioanalyzer. 
- LFM designs and fabricated by Mesa Tech International (NASBA reaction products also tested on the array). 

Results

- Successfully created primers for each species, combined in a primer cocktail that can be used for amplification of all targeted species (Figs. 3 & 4). 
- Target sequences used to design capture and reporter probes for LFMs at Mesa Tech International. Several rounds of optimization and probe re-design have produced successful discrimination among most species with limited cross hybridization (Fig. 4). 

Next Steps

- Use LFM and beacon on mixed target samples to investigate optimization of beacon performance 
- Sensitivity testing with seeded seawater 

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References