

2010 Ocean Sciences Meeting

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Rapid detection of *Pseudo-nitzschia* phylotypes using DNA microarrays

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The diatom genus *Pseudo-nitzschia* contains several species capable of producing the neurotoxin, domoic acid. The morphological differences that distinguish among toxin-producing versus non-toxic species are often too small to detect by light microscopy, requiring the laborious techniques of scanning and transmission electron microscopy. Molecular approaches offer the possibility of detecting species of interest by targeting distinct genetic sequences particular to those taxa. Two requirements must be met: (1) genetic sequences must be sufficiently variable in order to make distinctions among species and (2) morphology, sequences, and capability of toxin production must be in agreement. Using all publicly available sequences for the internal transcribed spacer (ITS) region between the 18S and 5.8S rRNA genes of *Pseudo-nitzschia* spp., we generated a set of probes to evaluate diversity among *Pseudo-nitzschia* phylotypes from the Pacific Northwest coastal margin. Aboard research cruises we used DNA microarrays with a set of 307 probes for 96 ITS sequences to generate phylotype profiles for *Pseudo-nitzschia*. We first identified samples with detectable *Pseudo-nitzschia* cells using an imaging flow cytometer and then extracted DNA from concentrated samples from those sites. We PCR-amplified ITS sequences from the purified DNA to serve as targets, biotinylated them, and hybridized the labeled targets onto the microarrays for electrochemical detection of probe-target hybridization using the ElectraSense system (CombiMatrix Corp.). The ElectraSense detection principle relies on a secondary enzymatic redox reaction that creates the current flux when a DNA target hybridizes to oligonucleotide probes attached to the electrode surface. The data provide direct numeric quantification of the hybridization signals for all probe-target interactions. We were able to process samples within 6-8 h of collection aboard the research vessel, and to detect a range of *Pseudo-nitzschia* phylotypes that was consistent with observations from the imaging flow cytometer. A comparison of *Pseudo-nitzschia* phylotype diversity over different seasons revealed differences in complexity that reflected environmental conditions; using DNA microarrays can provide a rapid means to assess these relationships, if the phylotypes profiles accurately reflect taxonomic assignment. In order to reliably assign phylotypes to species of interest, we isolated and grew cultures of *Pseudo-nitzschia* in the laboratory to create fingerprints of known species. Identity was confirmed by electron microscopy and 18S rDNA sequence analysis. While this method of taxonomic identification does not indicate whether the cells are toxic or non-toxic, it nonetheless provides a rapid means to determine whether the species is one that is capable of producing toxins, and thus reduces the need to determine toxicity for species that are likely harmless, potentially improving time and cost efficiency for coastal harmful algal bloom monitoring.

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