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Phytochip: Development of a DNA-microarray for rapid and accurate identification of *Pseudo-nitzschia spp* and other harmful algal species

Charlotte Noyer[1,2,3,4], Anne Abot[2,3,4], Lidwine Trhouilh[2,3,4], Véronique Anton Leberre[2,3,4], Catherine Dreanno[1]

1. *IFREMER, Centre Bretagne, Laboratoire Detections, Capteurs et Mesures, F-29280 Plouzané, France*
2. *Université de Toulouse, INSA, UPS, LISBP, F-31077 Toulouse, France*
3. *INRA, UMR792, Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France*
4. *CNRS, UMR5504, F-31400 Toulouse, France*

Abstract:

Detection of harmful algal blooms has become a challenging concern because of the direct impacts on public health and economy. The identification of toxic dinoflagellates and diatoms in monitoring programs requires an extensive taxonomic expertise and is time consuming. Advances in molecular biology have allowed the development of new approaches, more rapid, accurate and cost-effective for detecting these microorganisms. In this context, we developed a new DNA microarray (called, Phytochip) for the simultaneous detection of multiple HAB species with a particular emphasis on *Pseudo-nitzschia* species. Oligonucleotide probes were designed along the rRNA operon. After DNA extraction, the target rDNA genes were amplified and labeled using an asymmetric PCR; then, the amplicons were hybridized to the oligonucleotide probes present on the chips. The total assay from seawater sampling to data acquisition can be performed within a working day. Specificity and sensitivity were assessed by using monoclonal cultures, mixtures of species and field samples spiked with a known amount of cultured cells. The Phytochip with its 81 validated oligonucleotide probes was able to detect 12 species of *Pseudo-nitzschia* and 11 species of dinoflagellates among which were 3 species of *Karenia* and 3 species of *Alexandrium*. The Phytochip was applied to environmental samples already characterized by light microscopy and cloned into DNA libraries. The hybridizations on the Phytochip were in good agreement with the sequences retrieved from the clone libraries and the microscopic observations. The Phytochip enables a reliable multiplex detection of phytoplankton and can assist a water quality monitoring program as well as more general ecological research.

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