DEVELOPMENT OF A MICROARRAY-BASED ASSAY FOR THE DETECTION OF HARMFUL DINOFLAGELLATES

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Abstract

The high throughput, automation possibility and specificity of microarray-based detection assay, makes this technology very promising for qualitative HAB (harmful algal bloom) detection. In this study, we set up an assay based on the use of microarrays for the simultaneous detection of 9 dinoflagellate species/clades in Mediterranean Sea. The array format, hybridization and staining protocols were optimized, and the probe specificity and sensitivity were evaluated either with cultured microalgae or field samples, demonstrating the applicability of this assay for molecular monitoring of toxic microalgae.

Keywords: Monitoring, Dinoflagellates, Biotechnologies

In the last years, several molecular methods having ribosomal DNA (rDNA) as target have been developed for monitoring HAB species (mainly dinoflagellates) [1]. In this context, the use of DNA microarrays represents a promising molecular approach, able to detect simultaneously a number of DNA sequences in an unknown sample. The application of microarray technology for the detection of HAB species has been illustrated by several recent publications [2, 3, 4, 5], in which the target DNA was represented by 18S and/or 28S rDNA genes and the length of the probes immobilized on a glass slide was between 18 and 25 nucleotides. It is also noteworthy that some authors, to reduce the number of false positives, adopted a hierarchical probe approach [2, 3, 4]. In this study, a microarray-based assay for the simultaneous detection and monitoring of 9 dinoflagellate species in the Mediterranean Sea was developed. The target dinoflagellates included *Alexandrium andersoni, A. tamarense ME, A. tamarense WE, A. catenella, A. minutum, A. taylori, A. pseudogoniaulax, Protoceratium reticulatum* and *Lingulodinium polyedrum*. The species-specific probes were 45-47 nucleotides in length, and were designed on the ITS1-5.8S-ITS2 rDNA region using the software Oligoarray 2.1. These probes were designed to identify selected dinoflagellate species in the Mediterranean Sea, plus several non-Mediterranean strains. Due to probe length and the rDNA region chosen, it was not possible to adopt a hierarchical probe approach. Then, to reduce the number of false positives, two specific probes were designed for each species. Moreover, a positive control probe and a negative control probe were included as microarray performance quality control. The microarray slide format consisted in 24 subarrays, each made of 18 columns and 22 rows. A single subarray contained 10 replicates of each probe (including positive and negative control probes) for a total of 200 spots, to maximize statistical significance of the results. The target rDNA region was PCR-amplified using conserved primers, and labeled by incorporation of dUTP-biotin. Detection of the hybridized PCR products was accomplished using Cy5-streptavidin. The hybridization protocol was optimized and the specificity and sensitivity of the method were tested using genomic DNA extracted from 20 cultivated strains. The value of mean background of the entire subarray plus three standard deviations was considered as threshold for a positive signal. The probe list and the hybridization specificity results are illustrated in Fig. 1.

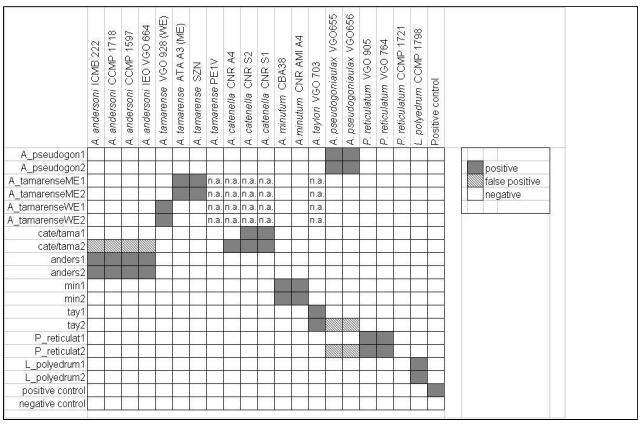

Fig. 1. Summary of specificity tests for the microarray probes (on the left). Target DNA consisted in the PCR-amplified ITS1-5.8S-ITS2 rDNA region of strains indicated in the upper part of the figure. n.a.: not available.

It is noteworthy that, when amounts of PCR product $\geqslant$100 ng of *A. andersoni* and *A. pseudogoniaulax* were used, a false positive signal appeared in probes cate/tama2, and tay2 / P_reticulat2, respectively. Nevertheless, concerning *A. pseudogoniaulax*, the false positive signals in probes tay2 and P_reticulat2 disappeared when amounts of PCR product $\leqslant$10 ng were used for hybridization. The false positive results observed in probes cate/tama2, tay2 and P_reticulat2 did not affect the reliability of the assay because the fluorescence on probes cate/tama1, tay1 and P_reticulat1 was below the threshold. The assay specificity was maintained also mixing different PCR products in the same hybridization mixture or amplifying genomic DNA extracted from 4 different *Alexandrium* species. Concerning the sensitivity of the microarray system, the species-specific probes allowed to detect up to 2 ng of labeled PCR product amplified from a DNA extracted from pure cultures. The microarray potential was tested with net samples from northern Adriatic Sea, unspiked or spiked with known amounts of target dinoflagellate species. Results confirmed the specificity observed with previous experiments and revealed the presence of *A. pseudogoniaulax* cells in Northern Adriatic (confirmed by Utermohl counts) (Fig. 2). Moreover, using spiked samples, it has been established that the sensitivity of the assay in net samples reached 1 cell/ml, with some variability from one species to another. The optimized procedure of the assay is rapid (1 working day) and allows the processing of up to 24 samples on a single slide, making microarray application attractive for monitoring programs.

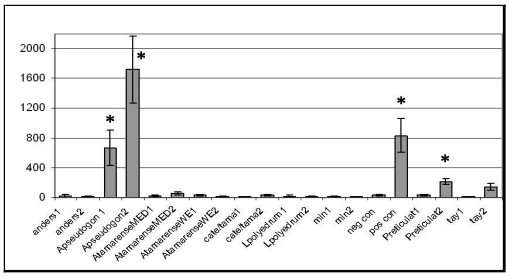

Fig. 2. Microarray analysis results of PCR products amplified from a net sample. The PCR-amplified positive control DNA (10 ng) was added in all hybridization mixtures. Asterisks indicate values above threshold

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