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The ALEX CHIP—Development of a DNA chip for identification and monitoring of *Alexandrium*

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Abstract

Harmful Algae Blooms (HABs) threaten humans, ecosystems, fishery, tourism, and aquaculture, and the occurrence of single cells in mixed phytoplankton assemblages is often difficult to detect. The genus *Alexandrium* has undergone steady taxonomic revision since its first description, and identification of its species has been confused because of overlapping morphological features and minute differences. The design of molecular probes from the 18S to 28S rDNA has shown great potential for distinguishing of species or even clades, but using these probes in a whole-cell hybridization format is tedious and time-consuming. Solid-phase methods, such as DNA microarrays, offer the potential to analyze multiple targets in a single experiment. This study describes the development of a DNA microarray for detection of several species belonging to the genus *Alexandrium*. Nine probes from other hybridization methods (fluorescence-*in-situ*-hybridization [FISH] and sandwich hybridization assay [SHA]) were tested on the microarray, and one new probe was developed for *Alexandrium minutum*. The specificity of the probes was tested by hybridization with 18S and 28S PCR-fragments from pure cultures and by analysis of filtered and spiked seawater samples from the Weser estuary (German Bight). Some published SHA and FISH probes did not work in a microarray format. A hybridization protocol was established, and the subset of the best performing probes for each species or clade was determined and recommended for classification and monitoring of field samples in the high throughput microarray format.

Keywords: *Alexandrium*; DNA microarray; Hybridization; Molecular probes; Paralytic shellfish poisoning (PSP)

1. Introduction

Harmful Algae Blooms (HABs) have a serious impact on public health and on the economic stability in many areas (Hallegraeff, 1993, 2003; Van Dolah, 2000; Hoagland et al., 2002). They threaten humans, complex ecosystems, and important economic areas with fishery, tourism, and aquaculture. Their frequency, intensity, duration, and geographic distribution seem to have increased the last decades (Scholin et al., 1994; Godhe, 2002; Hallegraeff, 2002). HABs can introduce several illnesses, and one of the worst is paralytic shellfish poisoning (PSP), which is caused by a group of neurotoxins, mainly saxitoxins (Hallegraeff, 1993). When contaminated fish or shellfish are ingested, these neurotoxins block the neural sodium channels in the human body (Taylor and Fukuyo, 1998). Some of the most important and thoroughly investigated PSP toxin producers can be found within the dinoflagellate genus *Alexandrium* (Balech, 1995; Cembella, 1998; Taylor and Fukuyo, 1998), although some non-toxic species are also present (Janson and Hayes, 2006).

The differentiation of *Alexandrium* species is difficult and tedious, because it mainly depends on minute morphological characteristics, e.g., fine thecal tabulation, chain formation, and cell shape (Balech, 1995). Furthermore, the different taxonomic patterns can vary with environmental conditions, and also morphological intermediate forms have been observed (Cembella and Taylor, 1985; Hallegraeff, 2003; Hosoi-Tanabe and Sako, 2005; John et al., 2005). Therefore, the exact species determination requires time and taxonomic expertise. The three species *Alexandrium tamarense* (Lebour) Balech, *Alexandrium catenella* (Whedon and Kofoid) Balech, and *Alexandrium fundyense* Balech are particularly demanding to differentiate because they are separated mainly by the presence or absence of a ventral pore and colony formation. They also share overlapping thecal characteristics. It has been shown that the strains of these species are related by geographic origin rather

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than by morphology and therefore, they are often referred to as the *A. tamarense* "species complex" (Cembella et al., 1987, 1988; Scholin et al., 1995; Medlin et al., 1998). The phylogeny of this species complex has been studied intensively (Scholin et al., 1994; Adachi et al., 1996; Medlin et al., 1998; Higman et al., 2001) and the geographic areas correspond to six different genetically distinct "ribotypes", based on the D1/D2 region of the 28S rRNA gene. The three toxic ribotypes are: the North American (NA), the Temperate Asian (TA) and the Tropic Asian (TROP) (John et al., 2005); members of the NA clade also have been found in Asian waters (Scholin et al., 1994), from the Orkney Islands (Medlin et al., 1998) and in South America (Persich et al., 2006). The non-toxic strains are: the Tasmanian (TASM), the Western European (WE) (Scholin et al., 1995) and the recently described Mediterranean (ME) clade (John et al., 2003b). The most characteristic feature for identification of *Alexandrium ostenfeldii* (Paulsen) Balech and Tangen is the shape of its first apical plate and its large ventral pore (Balech, 1995). It is the least toxic of the toxic species in *Alexandrium* (Cembella et al., 1987, 1988; Hansen et al., 1992), but highly toxic strains have also been found, and they produced both PSP toxins (Mackenzie et al., 1996) and spirolides (Cembella et al., 2000, 2001; Hallegraeff, 2002). For the identification of *Alexandrium minutum* Halim, minimal details of the apical tabulation (Taylor et al., 1995; Hallegraeff, 2002) and the characteristic ventral pore (Faust and Gulledge, 2002) are used, but strains without ventral pores have also been reported (Taylor et al., 1995; Vila et al., 2005). *A. minutum* also produces PSP toxins and other toxins (Taylor and Fukuyo, 1998; Chen and Chou, 2002; Nascimento et al., 2005).

All these toxic *Alexandrium* species are distributed worldwide, mainly in coastal areas (Balech, 1995; Taylor et al., 1995; Faust and Gulledge, 2002; Hallegraeff, 2002; Lundholm and Moestrup, 2006) and co-occur with non-toxic species. Therefore a reliable detection of these species is highly desirable. Traditional microscope based techniques are tedious and time-consuming, but molecular methods, especially the utilization of molecular probes, have shown great potential for *Alexandrium* species identification. One great advantage is that they are based on genetic features rather than on morphological characteristics (John et al., 2003a, 2005; Anderson et al., 2005; Metfies et al., 2005). A further promising molecular approach has been presented by DNA microarrays, which are applied generally for gene expression (Schena et al., 1995, 1996), but have also been used with oligonucleotide probes of conserved genes for species identification at all taxonomic levels (Metfies and Medlin, 2004; Loy et al., 2005; Ki and Han, 2006; Medlin et al., 2006a; Peplies et al., 2006). The technique is based on a minimized, but high throughput form of a dot blot through application of sequences or probes in an ordered array on the chip. The chip is made of glass and has special surface properties. The microarray offers the potential to facilitate the analysis of multiple targets from one sample in one experiment (Schena et al., 1995; Lockhart et al., 1996; DeRisi et al., 1997; Lockhart and Winzeler, 2000; Ye et al., 2001; Gentry et al., 2006; Metfies et al., 2006). A combination of molecular probes and DNA microarrays could serve as a rapid and reliable tool for detection of toxic microalgae and is not affected by environmental conditions or cell physiology.

This study evaluated and assessed the application of previously published probes to a DNA microarray. The probes target species of the genus *Alexandrium* and are specific in other methods. We developed a microarray (ALEX CHIP) to detect species of the genus *Alexandrium* in pure cultures and filtered seawater samples spiked with *Alexandrium* cells. The experiments showed that the microarray is a valid tool for monitoring of toxic microalgae.

2. Materials and methods

2.1. Culture conditions

The algal strains listed in Table 1 were cultivated under sterile conditions in seawater-based F2-media for *A. fundyense* (Guillard and Ryther, 1962), IMR-media for *A. tamarense* GTLI21, BAHME182, SZNB01, 08, 19 and 21 (Eppley et al., 1967) and K-media for all other species (Keller et al., 1987) at

Table 1
Algal cultures

Species	Strain (geographical clade)	Origin
A. ostenfeldii	CCMP1773	Limfjordan, Denmark, Hansen
A. ostenfeldii	K0324	Scandinavian Culture Centre for Algae and Protozoa, Denmark
A. minutum	AL3T	Gulf of Trieste, Italy, A. Beran
A. minutum	BAHME91	Biologische Anstalt Helgoland, Germany
A. fundyense	CA28 (NA)	Woods Hole, Oceanogr. Institution, D.M. Anderson
A. tamarense	BAHME225 (NA)	Biologische Anstalt Helgoland, Germany
A. tamarense	GTLI21 (NA)	Mud Creek, Moriches Bay, Long Island, USA
A. tamarense	31/9 (NA + WE)	Cork Habour, Ireland, W. Higman
A. tamarense	31/4 (WE)	Cork Habour, Ireland, W. Higman
A. tamarense	BAHME182 (WE)	Biologische Anstalt Helgoland, Germany
A. tamarense	UW42 (WE)	Belfast, UK, W. Higman
A. tamarense	SZNB01 (ME)	Gulf of Naples, Italy, M. Montresor
A. tamarense	SZNB08 (ME)	Gulf of Naples, Italy, M. Montresor
A. tamarense	SZNB19 (ME)	Gulf of Naples, Italy, M. Montresor
A. tamarense	SZNB21 (ME)	Gulf of Naples, Italy, M. Montresor