Harmful Algae 32 (2014) 40–48

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Monitoring a toxic bloom of *Alexandrium minutum* using novel microarray and multiplex surface plasmon resonance biosensor technology

Gary R. McCoy [a,*], Sara McNamee [b], Katrina Campbell [b], Christopher T. Elliott [b], Gerard T.A. Fleming [a], Robin Raine [c]

[a] The Ryan Institute, School of Natural Sciences (Microbiology), National University of Ireland, Galway, Ireland
[b] Institute for Global Food Security, School of Biological Sciences, Queen's University, Stranmillis Road, Belfast, UK
[c] The Ryan Institute, National University of Ireland, Galway, Ireland

ARTICLE INFO

Article history:
Received 21 August 2013
Received in revised form 11 December 2013
Accepted 11 December 2013

Keywords:
Alexandrium
Biosensor
Microarray
MIDTAL
RNA
Saxitoxins

ABSTRACT

Blooms of *Alexandrium* occur annually during the summer months in the North Channel of Cork Harbour on the south coast of Ireland. This study monitored an extensive bloom of the toxin producing *Alexandrium minutum* during the summer of 2011 with the use of the MIDTAL (Microarrays for the Detection of Toxic Algae) microarray and a prototype multiplex surface plasmon resonance (multi SPR) biosensor. Microarray signal intensities and toxin results from three testing platforms of the prototype multi SPR biosensor, commercial (CER) enzyme-linked immunosorbent assay (ELISA) and high performance liquid chromatography (HPLC) were compared against light microscopy counts. The main aim was to demonstrate the use of these methodologies to support national monitoring agencies by providing a faster and more accurate means of identifying and quantifying the harmful phytoplankton community and their toxins in natural water samples. Both the microarray signals and multi SPR biosensor results followed a significant trend with light microscopy results and both techniques indicated detection limits of <4000 cells of *A. minutum* in natural seawater samples.

1. Introduction

The dinoflagellate genus *Alexandrium* encompasses approximately 30 morphologically defined species of which at least half produce potent toxins (Balech, 1995; Anderson et al., 2012). These toxins bio-accumulate through the food chain, impacting humans, fish, birds and marine mammals on a global scale (Hallegraeff, 1993; Anderson et al., 2012). Saxitoxins and spirolides are the known toxins produced by this genus. Saxitoxin and its analogues are the causative agents of the human illness paralytic shellfish poisoning (PSP), a condition that can be potentially fatal following ingestion of contaminated shellfish (Huang et al., 1996; Anderson et al., 2012). It is often difficult to discriminate between armoured dinoflagellate species and it requires a high degree of skill using traditional light microscopy (LM) methods. Moreover, it is challenging to discriminate between morphologically similar but genetically dissimilar strains, such as toxic and non-toxic forms of the same species or groups of monophyletic clades (Lilly et al., 2005, 2007). A variety of molecular methods have been adapted for the detection and quantification of *Alexandrium* species with gene probes. Fluorescent *in situ* Hybridisation (FISH) permits the selective detection of the genus *Alexandrium*, using oligonucleotide probes within a whole-cell format by means of fluorescence microscopy (Touzet et al., 2007; Tang et al., 2012). A sandwich hybridisation assay, involving cell lysis with two hybridisation reactions, has proved useful in obtaining near real-time mapping of the distribution of *Alexandrium* species when used onboard a ship (Diercks et al., 2008). Quantitative real-time PCR (qRT-PCR) can provide accurate and reproducible quantification of gene copy formation during exponential phase of the reaction (Galluzzi et al., 2004; Touzet et al., 2009; Erdner et al., 2010; Toebe et al., 2013). Further advances have led to the development of

Abbreviations: PSP, paralytic shellfish poisoning; FISH, Fluorescent *in situ* Hybridisation; qRT-PCR, quantitative real-time polymerase chain reaction; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; MIDTAL, Microarrays for the Detection of Toxic Algae; multi SPR, multiplex surface plasmon resonance; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; Poly (dT) tail, poly (deoxythymidylic) tail; BCP, 1-bromo-3-chloro-propane; S/N ratio, signal-to-noise ratio; STX, saxitoxin; dc STX, decarbamoyl saxitoxin; NEO, neosaxitoxin; dcNEO, decarbamoyl neosaxitoxin; GTX, gonyautoxins; dcGTX, decarbamoyl gonyautoxins.

* Corresponding author. Tel.: +353 091 493231; fax: +353 091 525005.
E-mail addresses: g.mccoy3@nuigalway.ie, gary.mccoy8@gmail.com (G.R. McCoy).

1568-9883/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.hal.2013.12.003

DNA-biosensors for electrochemical detection of phytoplankton and their toxins (Metfies et al., 2005).

Microarrays are the state of the art technology in molecular biology for processing bulk samples for the detection of target RNA/DNA sequences. An RNA-based approach for species identification, using oligonucleotide probes that specifically target the 18S–28S rDNA domains from hierarchical groups down to the species level have been adapted for use with microarrays (Metfies and Medlin, 2004, 2008; Galluzzi et al., 2011; Gesher et al., 2008). Using the microarray technology in this way, the simultaneous analysis of 136 different probes and 4–8 replicates including several controls specific for a range of harmful phytoplankton species can be carried out using the recently developed MIDTAL microarray (Lewis et al., 2012; Kegel et al., 2013a).

A novel multiplex optical surface plasmon resonance (multi SPR) prototype biosensor has been applied to the analysis of marine biotoxins (Campbell et al., 2011). Using this approach, the identification of domoic acid, okadaic acid and paralytic shellfish toxins using a single multi-biosensor chip is now possible. This method has been tested in parallel with the enzyme-linked immunosorbent assay (ELISA) and high performance liquid chromatography (HPLC) to verify the presence or absence of these toxins in seawater samples (McNamee et al., 2013).

The North Channel of Cork Harbour on the south coast of Ireland was chosen for a sampling survey of *Alexandrium* spp. in 2011. In this location, PSP producing *Alexandrium minutum* blooms occur regularly during the summer months, including the summer of 2011 (Touzet et al., 2007). This paper presents the results of inter-comparisons between light microscopy counts and microarray results using the MIDTAL microarray, as well as PSP toxin data derived from the multi SPR method and high performance liquid chromatography (HPLC) (Lewis et al., 2012; McNamee et al., 2013; Kegel et al., 2013a). The aim of both these newly developed technologies is the provision of new methods to support toxic algal monitoring, thus contributing to the safeguarding of human health and supporting common fisheries policies across Europe and the US (Lewis et al., 2012).

2. Materials and methods

2.1. Sampling site and field sampling

Cork Harbour located on the south coast of Ireland is separated by Great Island, north of which is an eastern arm of the harbour known as the North Channel (Fig. 1A). The North Channel was the main area of sampling. At least two stations from locations N/O, P, and R (Fig. 1B) were sampled every week between 25 May and 13 July 2011 (Table 1). At each sampling site 50 ml seawater sample from discrete depths were placed in 50 ml cell culture bottles and preserved with 0.4 ml of unacidified Lugol's Iodine (Throndsen, 1978). The Lugol's preserved samples were kept in the dark until used for cell count determination, which was performed using an inverted microscope (Olympus CKX-41) following the procedure described in McDermott and Raine (2010). Water samples for RNA analysis were pre-filtered through a 150 μm mesh and then filtered through a 1 μm pore-size nitrocellulose filter (25 mm diam.). A total volume of 200–250 ml was filtered due to relatively high suspended matter found in the North Channel (Table 1). All filters for RNA analysis were immediately immersed in 1 ml of TRI Reagent (Ambion) contained in 2 ml screw cap tubes, kept at 4 °C during sampling and within 6 h of sampling stored at −80 °C on return to the laboratory. Water samples (0.6–1.0 L) for toxin analysis were filtered through glass fibre (Whatman GF/F, 47 mm diam.) filters (Table 1). These filters were stored separately at −20 °C in 2 ml screw cap tubes. Water temperature at 0.5 m off the sea bed was recorded hourly using a TidBit temperature sensor

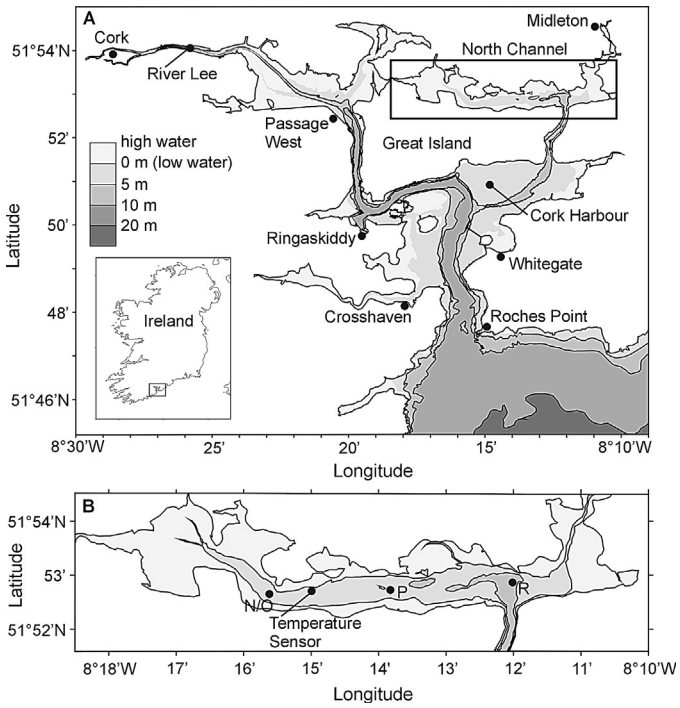

Fig. 1. (A) Map of Cork Harbour showing the location of the North Channel. (B) Sampling station positions in the North Channel (N/O, P, and R) and the temperature sensor position which was moored at this location throughout the survey period.

(HOBOware) moored between stations N/O and P throughout the sampling period (Fig. 1B). Temperature and salinity profiles were also measured *in situ* using a temperature salinity probe (WTW, 197i). Tidal ranges were derived from published tide tables.

2.2. Microarray design

Oligonucleotide probes routinely used for FISH were modified and adapted to the microarray, newly designed probes were also developed with the open software package ARB (Ludwig et al., 2004). The oligonucleotides including the positive and negative controls were synthesised (Eurofins MWG Operon or Thermo Fisher Scientific, Ulm, Germany) with a C6 aminolink at the 5′ end of the molecule. The probes on the second generation chip had a length between 18 and 25 nucleotides, and a further 15 nucleotide poly deoxythymidylic (dT) tail following the amino (NH_2) link at the 5′ end was subsequently added for the 3rd generation chip. The addition of an Amino C6 and Poly-T (15 nt) spacer was to lower cross reactivity between probes on the chip along with more stringent washing steps (Kegel et al., 2013a). The MIDTAL microarray probe sequences are patent pending and the entire hybridisation kit including the array and all necessary reagents are commercially available from Microbia Environement (France; contact@microbiaenvironement.com). The *Alexandrium* specific probes were originally based on sequences described by Miller and Scholin (1998), Guillou et al. (2002), John et al. (2003) and Kegel et al. (2013b) (Table 2). Duplicate arrays were spotted with 4–8 replicates of 136 different probes and as well as three negative controls (NEGATIVE1_dT, NEGATIVE2_dT, NEGATIVE3_dT), one positive control (TATA box protein), a Poly-T-Cy5 spotting control, and three internal controls (DunGS02_25, DunGS02_25_dT and DunGS05_25_dT for *Dunaliella tertiolecta*). After spotting, slides were incubated for 30 min at 37 °C and then stored at −20 °C. A list of the probes and targeted taxon made from the 18S or 28S rRNA gene to form the third generation of the MIDTAL microarray can be found in Kegel et al. (2013a).