Toxicon 122 (2016) 54–66

Contents lists available at ScienceDirect

Toxicon

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

Algal toxins and producers in the marine waters of Qatar, Arabian Gulf

Abdulrahman Al Muftah [a], Andrew I. Selwood [b], Amanda J. Foss [c], Hareb Mohammed S.J. Al-Jabri [a], Malcolm Potts [a], Mete Yilmaz [a, d, *]

[a] Qatar University, Department of Biological and Environmental Sciences, Doha, Qatar
[b] Cawthron Institute, Private Bag 2, Nelson, New Zealand
[c] GreenWater Laboratories/CyanoLab, 205 Zeagler Drive, Palatka, FL, 32177, USA
[d] Bursa Technical University, Department of Bioengineering, 16310, Bursa, Turkey

ARTICLE INFO

Article history:
Received 25 June 2016
Received in revised form
18 September 2016
Accepted 20 September 2016
Available online 21 September 2016

Keywords:
Arabian Gulf
Amnesic shellfish toxin
Cyclic imine
Diarrhetic shellfish toxin
Paralytic shellfish toxin
Pinnatoxin
Portimine
Vulcanodinium rugosum

ABSTRACT

Harmful Algal Bloom species are ubiquitous and their blooms occur in the Arabian Gulf. In this study, two cruises were performed in 2012 and 2013 to collect phytoplankton samples from 4 sites in the Arabian Gulf. Toxin analyses of phytoplankton samples for 32 algal toxins from 5 different toxin groups were conducted on the samples using both enzyme linked immunosorbent assay (ELISA) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Results demonstrated, for the first time, the presence of paralytic shellfish toxins (PSTs), diarrhetic shellfish toxin (DST), amnesic shellfish toxin (AST), cyclic imines (CIs) and polyether-lactone toxins in freeze-dried phytoplankton samples. Four *Vulcanodinium rugosum* cultures were established from field samples and these proved to contain between 603 and 981 ng pinnatoxin (PnTx) H per mg dry weight in addition to being positive for portimine. These strains from Qatar clustered with strains from Japan and Florida based on large subunit rRNA and rRNA internal transcribed spacer gene sequences.

1. Introduction

The Arabian (Persian) Gulf (hereafter the Gulf) is a partially-enclosed shallow sea at the northwest part of the Indian Ocean and connects to the ocean through the narrow Strait of Hormuz (Reynolds, 1993). The Gulf is a major, and for some countries, the only drinking/municipal water and seafood source in the region. In addition to naturally high temperatures and salinity levels, the Gulf has been under serious anthropogenic environmental disturbances, including coastal dredging, harmful effluents, overfishing, reduced freshwater input, and intense maritime traffic resulting in the transfer of exotic organisms from ballast water (Sheppard et al., 2010). Some of the aforementioned environmental disturbances have been linked to increased occurrences of dinoflagellates and Harmful Algal Blooms (HABs) in the Gulf (Richlen et al., 2010; Sheppard et al., 2010).

HABs have the potential to produce a wide variety of toxins in marine waters, including, paralytic shellfish toxins (PSTs), diarrhetic shellfish toxins (DSTs), polyether-lactone toxins, amnesic shellfish toxin (AST) and cyclic imines (CIs). PSTs are a class of neurotoxic alkaloids (57 analogs) that can be trophically transferred in the food web through vector organisms, such as bivalves and crustaceans (Wiese et al., 2010). Intoxication in humans can cause respiratory paralysis and death in severe cases (Cusick and Sayler, 2013; Wiese et al., 2010). To date, PSTs have been detected in some freshwater cyanobacteria (Foss et al., 2012) in addition to some marine dinoflagellate genera, including *Alexandrium*, *Pyrodinium* and *Gymnodinium* (Wiese et al., 2010).

DSTs are acidic polyether toxins including okadaic acid (OA) and dinophysistoxins (DTX 1, DTX 2 and their derivatives), presenting gastrointestinal symptoms including diarrhea and vomiting when consumed (Van Dolah, 2000). These toxins have been identified in marine dinoflagellate genera including *Dinophysis* and *Prorocentrum* (Fux et al., 2011; Reguera et al., 2014; Vale et al., 2009). *Dinophysis* species are also the only known producer of the pectenotoxins (PTXs), a group of poly-ether lactone toxins. PTXs exhibit

* Corresponding author. Bursa Technical University, Department of Bioengineering, 16310, Bursa, Turkey.
E-mail addresses: aralmuftah@qu.edu.qa (A. Al Muftah), andy.selwood@cawthron.org.nz (A.I. Selwood), amandafoss@greenwaterlab.com (A.J. Foss), h.aljabri@qu.edu.qa (H.M.S.J. Al-Jabri), mete.yilmaz@btu.edu.tr (M. Yilmaz).

http://dx.doi.org/10.1016/j.toxicon.2016.09.016
0041-0101/

hepatotoxicity in mice after intraperitoneal injection and are not considered diarrhetic, but are commonly grouped with the OA-group of toxins (Miles et al., 2004).

Amnesic shellfish poisoning is caused by the neurotoxin domoic acid (DA) through vector organisms such as shellfish or fish. DA is a tricarboxylic amino acid and glutamate receptor agonist (Van Dolah, 2000), causing calcium toxicity in neurons (Bejarano et al., 2008). Intoxication symptoms in humans include gastrointestinal problems and neurological disorders, such as memory loss, hence the name "amnesic shellfish poisoning" (Bejarano et al., 2008). DA is produced by several *Pseudo-nitzschia* species and *Nitzschia navis-varingica* (Kotaki et al., 2000).

CIs are a group of marine shellfish toxins with a common macrocycle structure and a cyclic imine moiety. CIs include the pinnatoxins (PnTxs), portimine, pteriatoxins, gymnodimines (GYMs), prorocentrolides, spiro-prorocentrimine, and spirolides (SPX) (Selwood et al., 2010). Since the compounds have similar chemical structures and toxicity, they have been grouped together. Generally, the CIs have the ability to inhibit acetylcholine receptors resulting in a wide range of neurotoxic symptoms (Bourne et al., 2010; Hellyer et al., 2015; Kharrat et al., 2008; MacKenzie et al., 2005; Munday et al., 2012, 2004; Selwood et al., 2013; Wandscheer et al., 2010). CIs are produced either by dinoflagellates or through shellfish metabolism. GYM has been isolated from the dinoflagellate *Karenia selliformis* (Miles et al., 2003, 2000). Interestingly, in 1999, a bloom of this species in the Kuwait bay was implicated in kills of wild and aquacultured fish, but toxins were not measured during that study (Heil et al., 2001). PnTxs, to date, have only been detected in the dinoflagellate *Vulcanodonium rugosum* (Hess et al., 2013; Rhodes et al., 2011, 2010; Selwood et al., 2014; Smith et al., 2011; Zeng et al., 2012). Portimine has also only been detected in *V. rugosum* cultures (Selwood et al., 2013).

Although routine monitoring of phytoplankton and HAB species does not currently exist in most of the Gulf, scattered short term studies provide some information on the spatial and temporal distribution of phytoplankton. Phytoplankton productivity is highest in the northern Gulf due to nutrients supplied by river discharges (Sheppard et al., 2010). Productivity decreases, but species diversity increases towards south (Subba Rao and Al-Yamani, 1998). Tropical diatoms and dinoflagellates dominate the reported phytoplankton species in the Gulf, with a few additional cyanobacteria species (e.g. *Trichodesmium* [Al-Muftah, 1991; Polikarpov et al., 2016; Subba Rao and Al-Yamani, 1998];). Primary production in the marine waters surrounding the Qatari Peninsula is low, but higher on the eastern coast and in the winter (Nour El-Din and Al-Khayat, 2005; Quigg et al., 2013). Although Dorgham and Al-Muftah (1986), and Al-Muftah (1991) identified over 250 species of phytoplankton along the Qatari coastline, Nour El-Din and Al-Khayat (2005) and Quigg et al. (2013) identified around 100 species; all dominated by diatoms.

The first HAB leading to fish mortalities in the Gulf was reported in 1999 from Kuwaiti coastal waters, where *Karenia selliformis* numbers reached 6×10^6 cells L^{-1}. This bloom resulted in kills of wild and aquacultured fish (Heil et al., 2001). Two years later a *Ceratium furca* bloom was blamed for Sea Bream mortalities in net pens, in the Kuwait bay (Glibert et al., 2002). Investigations of harmful algal species in the area during and after fish kills revealed the presence of high numbers of *Gymnodinium catenatum*, *Gyrodinium impudicum* and *Pyrodinium bahamense* var. *bahamense*, along with lower numbers of *Alexandrium* spp. and *Karenia* sp. Fish and shellfish were tested for saxitoxins and brevetoxins. Only the clam tissues were positive for saxitoxins, measuring 1.26 μg STX eq 100 g^{-1} via a receptor binding assay (Glibert et al., 2002). Blooms of other potentially harmful species were also reported from Kuwait marine waters in 2000, including *Trichodesmium erythraeum* (10^8 cells L^{-1}), *Pseudo-nitzschia seriata* (10^9 cells L^{-1}), *Prorocentrum* spp. (4.3×10^6 cells L^{-1}) and *Gymnodinium* spp. (2.9×10^6 cells L^{-1}) (Al-Yamani et al., 2012). A cruise covering 43 sites in the Arabian Gulf between February and March 2006 illustrated the dominance of gymnodinoid dinoflagellates followed by cryptophytes, diatoms and other dinoflagellate groups. Cell numbers exceeding 10^6 cells mL^{-1} were recorded at the central Gulf off Qatar (Polikarpov et al., 2016).

The most notable HAB event occurred between 2008 and 2009, when an 8-month bloom of the toxic dinoflagellate species *Cochlodinium polykrikoides* persisted in the coastal waters of Qatar, United Arab Emirates (UAE) and Oman resulting in massive die-offs of fish, marine mammals and coral reefs. The event also led to closures of desalination plants (Al-Azri et al., 2014; Richlen et al., 2010; Zhao and Ghedira, 2014).

Although potentially toxic dinoflagellate and diatom species and their blooms have been reported in the Gulf, to the authors' knowledge, Glibert et al. (2002) was the only study to measure algal toxins. Therefore the focus of this study was to investigate the presence of a variety of algal toxins in the Gulf and in dinoflagellate cultures isolated from the Qatari marine waters. Thirty-two toxins from 5 algal toxin groups were analyzed and included PSTs, DSTs, polyether toxins, AST and CIs. Qualitative algal identification and culture isolations were also conducted in order to help elucidate the source of the toxins detected in the Gulf.

2. Materials and methods

2.1. Study area and sampling

The Gulf is 1000 km long, 338 km wide (Fig. 1, inset), with an average depth of about 36 m (Reynolds, 1993). The State of Qatar is a peninsula located in the southwestern Gulf covering an area of approximately 11,500 km^2 with over 560 km of contiguous coastline (Fig. 1). In this present study two cruises were carried out on R/V Janan of Qatar University on October 18, 2012 and November 13, 2013 for the sampling stations shown in Fig. 1. These stations were chosen to sample both near-shore and off-shore marine waters of Qatar. Water depths of stations 1 to 4 were 10 m, 24 m, 28 m and 34 m, respectively. Vertical profiles of temperature, conductivity, pH and dissolved O_2 (DO) were obtained with YSI multiparameter probes connected to a YSI datalogger. Horizontal phytoplankton tows were performed at the surface with 20-μm and 50-μm phytoplankton nets for 10 min at each site, with a total of 7 samples collected over the study period. Samples for microscopic analyses were preserved with the addition of 5% (w/v) formalin or Lugol's solution. These were utilized for qualitative phytoplankton analyses as presence, or absence, at different sites on different sampling dates using a Leica upright microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Scanning Electron Microscopy (SEM) analyses were used to identify some dinoflagellates. For SEM, sample was washed with distilled water (5–7 times) and dehydrated through an ethanol series (50%, 70%, 90%, 100%), dried and coated with gold-palladium to examine on a Nova NanoSEM 450 (FEI, Hillsboro, Oregon, USA). Background in SEM photos of dinoflagellates was removed using Adobe Photoshop CC (Adobe Systems Incorporated, CA, USA). Taxonomic identifications were conducted according to various methods (Hallegraeff et al., 2010; Taylor, 1976; Tomas, 1997; Tregouboff and Rose, 1957; Wood, 1968). Phytoplankton tow samples (pooled 20-μm and 50-μm fractions) for toxin analyses were carried to the laboratory on ice, centrifuged in a Thermo Scientific SL40R (Thermo Scientific, Waltham, MA, USA) at 2400×*g* for 10 min, frozen and freeze-dried in a FreeZone 12 L Console Freeze Dry System (Labconco, Kansas City,