Chemosphere 183 (2017) 80–88

Contents lists available at ScienceDirect

Chemosphere

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

Occurrence of marine algal toxins in oyster and phytoplankton samples in Daya Bay, South China Sea

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HIGHLIGHTS

- *D. caudata* and *D. acuminata* complex were identified as Okadaic acid (OA)/pectenotoxin (PTX) related species.
- PTX2 was the predominant toxin in *D. caudata*, while OAs were related with *D. acuminata* complex.
- This paper is the first to report the detection of GYM, DA, and homoYTX in phytoplankton samples in Chinese coastal waters.
- GYM exhibited the highest frequency of positive detections in phytoplankton concentrates (13/17) and oysters (14/17).

ARTICLE INFO

Article history:
Received 4 January 2017
Received in revised form
12 April 2017
Accepted 11 May 2017
Available online 12 May 2017

Handling Editor: Jim Lazorchak

Keywords:
Shellfish toxins
Okadaic acid
Pectenotoxin
Dinophysis
Domoic acid
Pseudo-nitzschia

ABSTRACT

The occurrence and seasonal variations of marine algal toxins in phytoplankton and oyster samples in Daya Bay (DYB), South China Sea were investigated. Two *Dinophysis* species, namely, *D. caudata* and *D. acuminata* complex, were identified as Okadaic acid (OA)/pectenotoxin (PTX) related species. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis demonstrated that 2.04–14.47 pg PTX2 per cell was the predominant toxin in single-cell isolates of *D. caudata*. *D. acuminata* was not subjected to toxin analysis. The occurrence of OAs in phytoplankton concentrates of net-haul sample coincided with the presence of *D. accuminata* complex, suggesting that this species is most likely an OA producer in this sea area. OA, dinophysistoxins-1 (DTX1), PTX2, PTX2sa, gymnodimine (GYM), homoyessotoxin (homoYTX), and domoic acid (DA) demonstrated positive results in net haul samples. To our best knowledge, this paper is the first to report the detection of GYM, DA, and homoYTX in phytoplankton samples in Chinese coastal waters. Among the algal toxins, GYM demonstrated the highest frequency of positive detections in phytoplankton concentrates (13/17). Five compounds of algal toxins, including OA, DTX1, PTX2, PTX2sa, and GYM, were detected in oyster samples. DA and homoYTX were not detected in oysters despite of positive detections for both in the phytoplankton concentrates. However, neither the presence nor absence of DA in oysters can be determined because extraction conditions with 100% methanol used to isolate toxins from oysters (recommended by the EU-Harmonised Standard Operating Procedure, 2015) would likely be unsuitable for this water-soluble toxin. In addition, transformation of DA during the digestion process of oysters may also be involved in the negative detections of this toxin. GYM exhibited the highest frequency of positive results in oysters (14/17). OAs were only detected in the hydrolyzed oyster samples. The detection rates of PTX and PTX2sa in oysters were lower than those in the net haul samples.

1. Introduction

Marine algal toxins are secondary metabolites produced by marine toxic algae. These toxins can accumulate in bivalves via

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http://dx.doi.org/10.1016/j.chemosphere.2017.05.067
0045-6535/

feeding processes and present health risks to humans if contaminated shellfish are consumed (Gerssen et al., 2010). In recent years, high contents of marine algal toxins (especially lipophilic marine toxins, LMTs) in shellfish have been frequently reported in different regions worldwide (Gerssen et al., 2010; Reguera et al., 2012). Typical LMTs include okadaic acid (OA) and its derivatives (dinophysistoxins, DTXs), pectenotoxins (PTXs), gymnodimine (GYM), azaspiracids (AZAs), yessotoxins (YTXs), and spirolides (SPXs). High levels of OA and DTXs can cause diarrhetic shellfish poisoning (DSP), with intoxication symptoms of diarrhea, nausea, vomiting, and abdominal pain (Reguera et al., 2014). AZAs cause adverse effects comparable with OA, including similar symptoms of diarrhea, nausea, and stomach cramps (Gerssen et al., 2010). Although no human intoxications caused by YTXs, PTXs, GYM and SPXs have been reported, these toxins have led to adverse effects on the health of mice (Aune et al., 2002; Miles et al., 2004; Munday et al. (2004); Gerssen et al., 2010; Reguera et al., 2014). For example, intraperitoneal and oral injected YTXs resulted in the tumidness of heart muscle of mice (Aune et al., 2002), and PTXs caused damage to their livers (Miles et al., 2004). Moreover, domoic acid (DA) is a crystalline water-soluble toxin produced by 27 algal species, including red alga *Chondria armata*, *Amphora coffeaeformis*, and *Nitzschia navis-varingica*, but mainly from 24 *Pseudo-nitzschia* species (reviewed by Lelong et al., 2012; Teng et al., 2015, 2016; Dao et al., 2015; Percopo et al., 2016), and is known to cause amnesic shellfish poisoning. The symptoms of DA intoxication include nausea, abdominal cramps, headaches, loss of memory, and even death (Lelong et al., 2012).

LMT distribution along the Chinese coasts has gained increasing attention in recent decades. Mouse bioassay (MBA) has been the official method for LMT detection in China since 1994 (Wu et al., 2015). Through this method, numerous studies have suggested that LMTs are widely spread in China (Jiang et al., 2014 and citations therein). However, MBA test is nonspecific and only provides a general response to toxicity for LMTs and other marine toxins. In recent years, liquid chromatography with tandem mass spectrometry (LC-MS/MS) has been used to monitor LMTs along the Chinese coasts. LC-MS/MS method can provide specific quantification of the full range of LMTs. For example, many LMTs, including OA, DTXs, PTXs, GYM and AZAs, are found in shellfish (Liu et al., 2011; Wu et al., 2015; Wang et al., 2016), seawater (Li et al., 2014b; Chen et al., 2017), and toxic algae (Luo, 2011; Li et al., 2015).

An incident associated with DSP toxins in China was reported in May 2011. More than 200 people in Ningbo, Zhejiang province, and Ningde, Fujian province, developed symptoms consistent with DSP (diarrhea, nausea, vomiting), following the consumption of *Mytilus galloprovincialis*, which were cultured in Fujian province. The contaminated mussels contain high concentrations of OA and DTX1, reaching 215 and 195 μg/100 g meat, respectively (Li et al., 2012). In spite of that the shellfish production contributes significant economic profits in China, however lack of monitoring and management programs for marine algal toxins leave consumers easily exposed to contaminated shellfish (Wu et al., 2015). Moreover, the distribution and occurrence of LMT-producing algae have increased along the Chinese coasts (Gao et al., 2010; Liu et al., 2011; Luo, 2011; Jiang et al., 2014; Li et al., 2015), although this could be partly due to the intensive monitoring programs. These phenomena highlight the importance of regular monitoring programs for these toxins in aquaculture zone in China.

Oyster farming is one of the largest coastal aquaculture industries in the subtropical sea areas along the coasts of Guangdong province, with an annual production close to one million metric tons (Jiang et al., 2016). Daya Bay, situated between Shenzhen and Huizhou city, near the Pearl River Estuary, is an important maricultural area of Guangdong province. Previous study showed a high positive detection rate (44%) for LMTs in shellfish collected from Daya Bay by using MBA method (Li et al., 2014a). The MBA method may be appropriate for monitoring seafood safety because it provides an overall response from co-extracted LMTs, while the information of toxin profiles and concentration can not be obtained. However, no investigation has been performed on LMTs in shellfish and algae (especially *Dinophysis*) using LC-MS/MS, which can give a full understanding of the content and composition of LMTs in Daya Bay. The present study aims to (1) examine the toxin-producing *Dinophysis* species and determine which toxins are present in picked single-cell isolates; (2) investigate the composition and temporal variations in LMTs in phytoplankton concentrates; and (3) examine the contamination levels of LMTs in oysters and the coupling relationship of LMTs between oysters and algal samples in Daya Bay.

2. Materials and methods

2.1. Sampling location

Daya Bay is situated in the southeast of Mainland China and is characterized by a subtropical climate. Its depth ranges between 6 and 15 m and covers an area of 650 km^2 at flood tide. The study area is located at Dapeng Cove, spanning approximately 4.5 km (N–S) by 5 km (E–W), in the southwest portion of Daya Bay (Fig. 1). The water depth is less than 10 m and tidal current velocities are low (<10 cm/s) in Dapeng Cove. The suspended longline oyster farm, located in the inner part of the cove, cultures *Crassostrea angulata* (Jiang et al., 2016).

2.2. Sampling strategy

In this study, a monthly investigation from July 2013 to December 2014 has been conducted. One sampling station with water depth around 6 m was chosen in the central area of the oyster farm. Phytoplankton samples were collected by a vertical net haul (diameter 30 cm, 20 μm mesh size) in the upper 3.0 m to obtain integrated samples of the whole water column. Three net hauls were obtained, mixed together (ca. 900 mL) and filled up to 1.0 L by filtered seawater. In all cases, sea water from net hauls was separated into 5 aliquots. Of these, three aliquots of 200 mL were filtered by GF/F filters (Whatman, 0.7 μm, 47 mm). The filters were folded and wrapped with aluminium foil, and stored in the liquid nitrogen for later toxin extraction and analysis (see Section 2.4 and 2.5). The 4th aliquot of 100 mL was preserved with Lugol's iodine (final concentration of 1%) for identifying and counting *Dinophysis* and *Pseudo-nitzschia* cells (see Section 2.3). The 5th aliquot (300 mL) was used for selecting single cells of *Dinophysis*. More than 100 cells of *D. caudata* were obtained during September, October, and November 2013, and January 2014. However, *Dinophysis* cells were not isolated during other periods. The isolated cells were stored in 1 mL of filtered seawater and stored under −80 °C before analysis.

A total of 17 oyster samples were collected monthly from floating culture rafts, within 50 m away from the water-sampling station. The oysters were cultured on suspended longline and attached on strings (2.5 m length), with a water depth of from ~0.5 to 3 m in the upper layer. A total of twenty-five oysters were picked from the string at intervals of about 10 cm, washed with clean water, kept in portable icy incubators below 4 °C, and transported to the lab within 24 h.

2.3. Microscopic observation of phytoplankton

The Lugol's iodine-fixed net haul samples were examined by light microscope (Olympus BH-2, Olympus, Tokyo, Japan). The