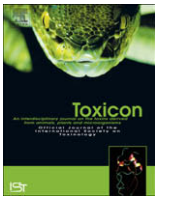
Toxicon 54 (2009) 364–367

Contents lists available at ScienceDirect

Toxicon

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



Short communication

Pectenotoxins and yessotoxin from Arica Bay, North Chile as determined by tandem mass spectrometry

Bernd Krock [a,*], Carmen Gloria Seguel [b], Katherinne Valderrama [b], Urban Tillmann [a]

[a] Alfred-Wegener Institut für Polar- und Meeresforschung, Am Handelshafen 12, 27570 Bremerhaven, Germany
[b] Universidad de Tarapacá, Avda. General Velásquez 1775, Arica, Chile

ARTICLE INFO

Article history:
Received 16 January 2009
Received in revised form 9 April 2009
Accepted 14 April 2009
Available online 23 April 2009

Keywords:
Toxic dinoflagellates
Dinophysis spp.
Protoceratium spp.
LC–MS/MS
Pectenotoxins
Yessotoxin
Arica Bay
Chile

ABSTRACT

Lipophilic phycotoxins were measured by tandem mass spectrometry coupled to liquid chromatography (LC–MS/MS) in size-fractionated plankton samples taken at five stations in Arica Bay, northern Chile in the southern summer 2007/2008. Pectenotoxins-2 (PTX-2), -11 (PTX-11), -2 seco acid (PTX-2sa) and yessotoxin (YTX) were identified by comparison of retention times and collision-induced mass spectra of certified standards and field sample extracts. This is the first report of PTXs and YTX from planktonic samples in Chilean coastal waters.

The rich biological productivity of the marine ecosystem Peru–Chile depends on the coastal upwelling system, which brings cold, nutrient rich, sub-surface waters to upper illuminated layer. This upwelling promotes high phytoplankton production, the nutritional basis for zooplankton and fish (Ulloa et al., 2001). Along the north Chilean coast this wind-driven upwelling occurs in pulses or events alternating with calms or northern winds (Pizarro et al., 1994). The upwelling leads to phytoplankton community succession, consisting mainly of diatoms, followed by dinoflagellates, which may dominate in warm periods (Avaria et al., 1982; Avaria and Muñoz, 1983; Avaria and Muñoz, 1985; Avaria and Muñoz, 1987). The dominance of diatoms together with high cell concentrations has been attributed to equatorial sub-surface waters, whereas the dominance of dinoflagellates is associated with the presence of warmer subtropical waters (Avaria and Muñoz, 1987).

Algal blooms in the area have been registered since 1956, but no associated human toxicity events have been observed to date. Among the bloom-forming species in northern Chile are the dinoflagellates *Prorocentrum micans*, *Gymnodinium* spp. *Glenodinium* spp. and the ciliate *Mesodinium rubrum* (Avaria et al., 1999). The presence of the latter is associated with upwelling processes, whereas dinoflagellate blooms in this region are related with the El Niño phenomenon (Rodríguez, 2004), characterized by relatively high water temperatures. In contrast, the La Niña phenomenon, which was present during the sampling period, is characterized by lower temperatures and lower dinoflagellate abundances.

The aim of this study was to conduct a preliminary survey of the coastal waters of Arica Bay, northern Chile for

* Corresponding author at: Alfred Wegener Institute for Polar and Marine Research, Ecological Chemistry, Am Handelshafen 12, 27570 Bremerhaven, Germany. Tel.: +49 471 4831 2055; fax: +49 471 4831 2115.
E-mail address: bernd.krock@awi.de (B. Krock).

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doi:10.1016/j.toxicon.2009.04.013

phycotoxins, since nothing is known about either the occurrence of toxigenic dinoflagellates or their respective toxins in the northern Chilean coastal waters. Knowledge of the occurrence of phycotoxins is important, because of increasing oyster culturing in this area.

Plankton samples were collected during three samplings at five stations in southern Arica Bay between 18°29.986′S–18°38.431′S and 70°19.463′W–70°21.492′W in the southern hemispheric summer on 17. Nov. 2007, 16. Dec. 2007 and 8. Jan. 2008. Water samples (60 L) were pumped from 0 (a), 10 (b) and 20 m (c) depth, respectively, and subsequently filtered *in situ* over Nitex mesh of various sizes (200, 70 and 20 μm). Residues on the 70 μm and 20 μm meshes were resuspended in a small volume of filtered seawater and then first centrifuged in test tubes for five minutes at 7 °C and 1500 × g. The pellets were transferred to micro tubes and again centrifuged for two min at 10,000 × g. The pellets were frozen at −20 °C and kept at this temperature until analysis.

Cell count samples for qualitative and quantitative determination of plankton species were collected with a sampling bottle (LabLine water sampler, model no. 4196) and fixed with formalin neutralized with borax. A total volume of 5.0–5.32 ml (depending on the chamber calibration) was counted by optical microscopy in five approximately 1 mL aliquots in Sedgewick-Rafter chambers and results expressed as means of the individual cell counts.

Mass spectrometric experiments were performed on an API-4000 Q-Trap (Applied Biosystems, Darmstadt, Germany), triple quadrupole-linear ion trap hybrid mass spectrometer coupled to an Agilent (Waldbronn, Germany) model LC 1100 liquid chromatograph. The method details are described in Krock et al. (2008).

For further identification of PTX-2, collision-induced mass spectra of the pseudo-molecular ion $[M + NH_4]^+$ m/z 876.5 were recorded in the enhanced production mode.

An MS^3 experiment for the unambiguous identification of YTX was performed in the negative mode and the pseudo-molecular ion $[M - H]^-$ m/z 1141.6 was fragmented by collision-induced dissociation (CID) in the collision cell. The most abundant fragment m/z 1061.6 was further fragmented in the linear ion trap.

The only detected toxin in the 20–70 μm size fractions were the pectenotoxins PTX-2 (max. 7604 pg L^{-1}) and in lesser amounts PTX-11 (max. 134 pg L^{-1}) and PTX-2sa (max. 39 pg L^{-1}), as well as YTX (max. 422 pg L^{-1}). The same toxins were also found in the 70–200 μm size fractions, but at far lower concentrations.

Further confirmation of the presence of PTX-2 in the plankton size fractions was obtained by a collision-induced dissociation (CID) experiment, which yielded identical fragment spectra for the PTX-2 standard and the putative PTX-2 in plankton field sample (Fig. 1).

Only *Dinophysis acuminata* (up to 2400 cells L^{-1}) and low amounts of *Dinophysis rotundata* (up to 200 cells L^{-1}), a possibly toxic *Dinophysis* species, were identified as known toxic dinoflagellate species in cell count samples. A *D. acuminata* bloom in Chilean coastal waters has been reported before (Blanco et al., 2007). The occurrence of *D. acuminata* in Chilean waters is not surprising, because

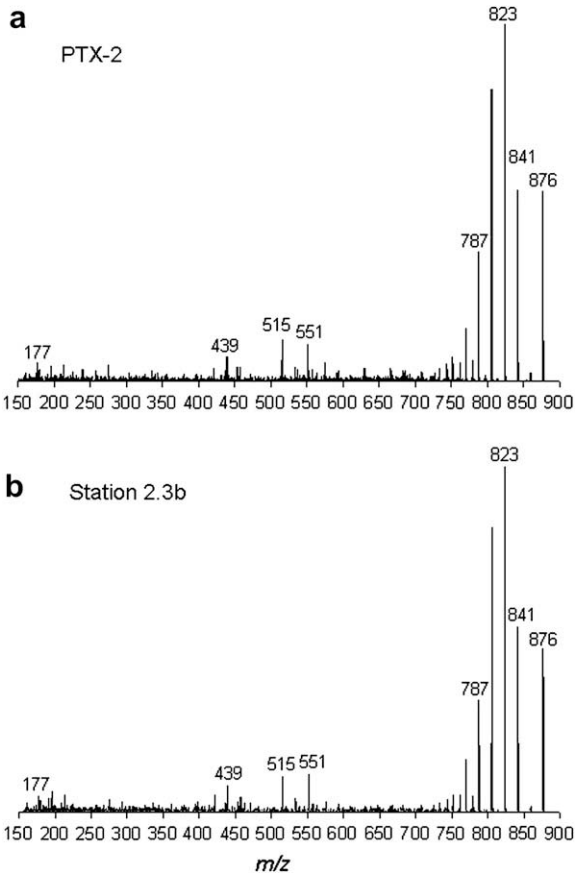

Fig. 1. Collision-induced dissociation (CID) mass spectra under positive ionization of the ion m/z 876. a) Standard pectenotoxin-2 and b) plankton 20–70 μm size fraction sampled at station 2.3b (10 m depth, 18°29.986′S latitude and 70°20.261′W longitude).

M. rubrum, which has been used as food source in the first successful attempts of culturing *Dinophysis* (Park et al., 2006; Kamiyama and Suzuki, 2009), forms recurrent blooms in Chilean waters (Avaria et al., 1999). Members of the genus *Dinophysis* are known PTX and dinophysistoxin (DTX) producers (including okadaic acid) (Blanco et al., 2007). The toxin profile found in the Arica Bay is consistent with other *D. acuminata* populations in the Pacific of the southern hemisphere. In New Zealand cell concentrates of *D. acuminata* produced much more PTXs than DTXs (PTX/DTX ratio >22) (MacKenzie et al., 2005) and in the coastal waters of Bahía Inglesa in the Chilean III Region no DTXs were detected in *D. acuminata* cell isolates (Blanco et al., 2007).

The other phycotoxin detected in the plankton field samples by LC–MS/MS in the multi-reaction monitoring (MRM) mode was YTX. The identity of YTX was confirmed by MS^3 experiments of YTX and a field sample extract (Fig. 2). YTX is distributed world wide and has been reported from Japan (Suzuki et al., 2007), New Zealand (Rhodes et al., 2006), South Africa (Krock et al., 2006), the US west coast (Howard et al., 2008), the North Sea (Aasen et al., 2005; Krock et al., 2008), the Mediterranean Sea (Ciminiello et al., 2002) and the Black Sea (Morton et al.,