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Short communication

Identification of yessotoxin in mussels from the Caucasian Black Sea Coast of the Russian Federation

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Abstract

The toxin load of shellfish hepatopancreas harvested from the Caucasian Black Sea Coast of the Russian Federation was investigated. The majority of the toxin load was shown to be yessotoxin (YTX), 45-hydroxy-yessotoxin (45-OH-YTX), and homoyessotoxin (homoYTX). Concurrent with the mussel intoxication, the dinoflagellates *Lingulodinium polyedrum* and *Gonyaulax spinifera* were found in high concentrations.
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Keywords: Black Sea; *Gonyaulax*; *Lingulodinium*; Phytoplankton; Yessotoxin

Yessotoxin (YTX) and its derivatives, 45-hydroxy YTX (45-OH-YTX), 45,46,47-trinor YTX, homo YTX, and 45-hydroxyhomo YTX (Satake et al., 1996, 1997a), are disulfated polyether lipophilic toxins originally isolated from Japanese scallops (Murata et al., 1987). This toxic polyether was produced by the dinoflagellates *Protoceratium reticulatum* (Claparhde and Lachmann) Buetschil (Satake et al., 1997b), *Lingulodinium polyedrum* (Stein) Dodge (Draisci et al., 1999), and *Gonyaulax spinifera* (Claparede and Lachmann) Diesing (Rhodes et al., 2006). YTX was originally associated with okadaic acid and pectenotoxins as a possible cause of diarrhetic shellfish poisoning (DSP). However, YTX, when administered to mice, does not present classic DSP-type symptoms. To date, this compound has been found in shellfish originating from Japan (Murata et al., 1987), Norway (Lee et al., 1988; Daiguji et al., 1998), Chile (Yasumoto and Takizawa, 1997), Italy (Satake et al., 1997a, b; Tubaro et al., 1998), New Zealand (Ramstad et al., 2001; Eiki et al., 2005), and the Russian White Sea (Vershinin et al., 2006a).

The aim of this work is to identify the presence of YTX and related derivatives from toxic mussels along the Russian Caucasian Coast and to investigate possible causative dinoflagellates.

Phytoplankton sampling was performed using a 10-μm-mesh plankton net at a Bolshoy Utrish site along the North-Caucasian Black Sea Coast on 1 August 2002 (Fig. 1). Live net samples were examined with a light microscope (Mikmed 2-2, LOMO, Sankt-Peterburg, Russia). For scanning

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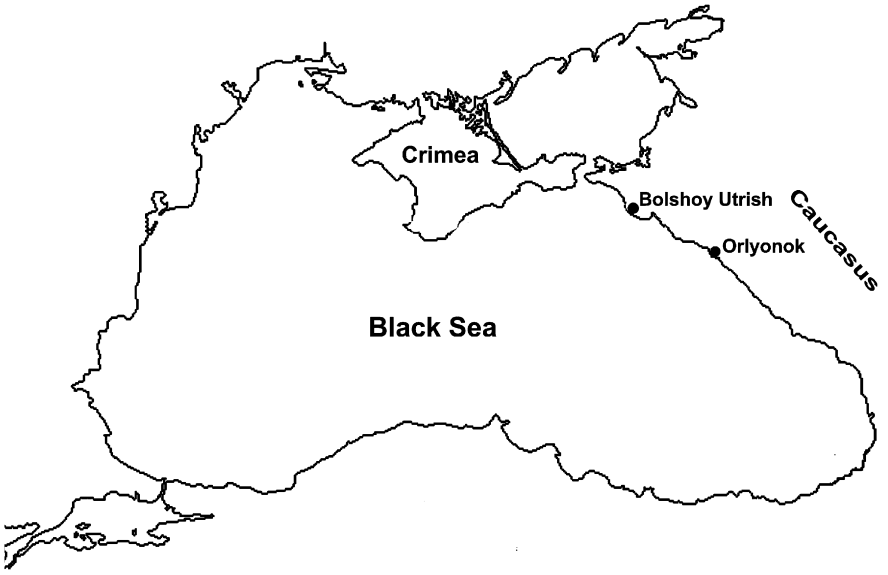

Fig. 1. Region map of the Black Sea.

electron microscopy (SEM), cells were desalted using a 10% step gradient of seawater to freshwater. Preparations were dehydrated using a series of ethanol (10–100%) and a series of hexamethyldisilazane (10–100%). The samples were coated with approximately 15 nm of gold using a Denton sputter-edge coater (Moorestown, USA). Samples were examined with a JEOL 5600LV (Tokyo, Japan) environmental SEM.

Mussels (*Mytilus galloprovincialis*) of 4–5 cm length were gathered at the time of phytoplankton sampling. Farmed mussels was collected at a depth of 1–10 m from collector ropes of the Utrish shellfish plantation. Wild mussels fouling the pier columns was sampled at Orlyonok. A 10 g sample of hepatopancreas tissue was added to triple the volume of methanol and frozen.

Analyses of lipophilic toxins were performed on an LC–MS/MS system consisting of an Agilent 1100 HPLC with a binary pumping system and an automated injector coupled to an API4000 mass spectrometer (PE-SCIEX, Concorde, Ont.) with a TurboSpray interface. The nebulizer gas temperature was set at 100 °C. Analyses were conducted with either selected reaction monitoring or product ion scanning modes. Negative ion mode was used for YTXs, while positive ion mode was used for other toxins. Product ion mass spectra were acquired by colliding the Q1-selected precursor ions with nitrogen in Q2 operated in radio frequency (rf)-only mode and scanning the second quadrupole mass spectrometer, Q3, from m/z 50–1200. The collision energy was set at 100 V for YTX and 35 V for other toxins.

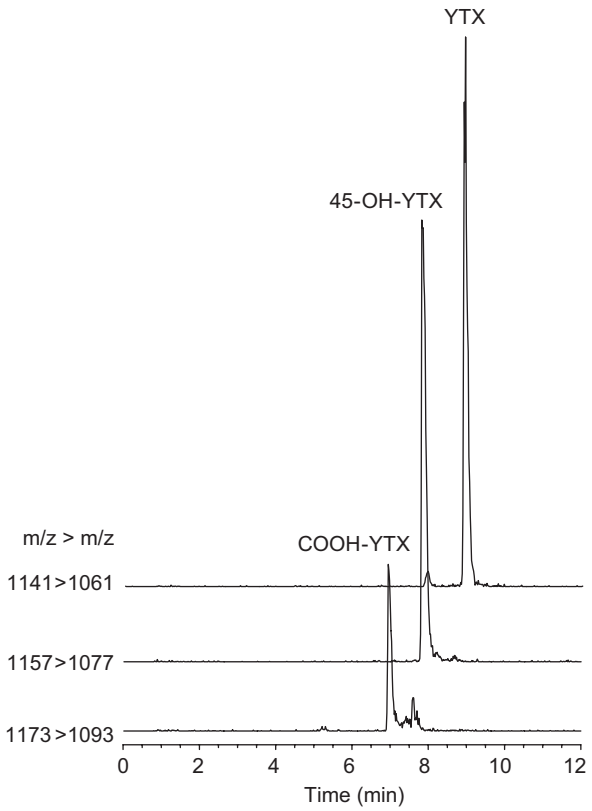

Fig. 2. LC–MS profile of yessotoxins in farmed mussel hepatopancreas.