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Toxin profile of *Alexandrium catenella* from the Chilean coast as determined by liquid chromatography with fluorescence detection and liquid chromatography coupled with tandem mass spectrometry

Bernd Krock [a,*], Carmen Gloria Seguel [b], Allan D. Cembella [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*

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Abstract

The profile of tetrahydropurine neurotoxins associated with paralytic shellfish poisoning (PSP) was determined from a Chilean strain of the marine dinoflagellate *Alexandrium catenella*. The toxin composition was compared with that of toxic shellfish, presumably contaminated by natural blooms of *A. catenella* from the same region in southern Chile. Ion pair-liquid chromatography with post-column derivatization and fluorescence detection (LC-FD) was employed for relative quantitative analysis of the toxin components, whereas unambiguous identification of the toxins was confirmed by tandem mass spectrometry (LC–MS/MS). In the dinoflagellate strain from Chile, the *N*-sulfocarbamoyl derivatives (C1/C2, B1) and the carbamoyl gonyautoxins GTX1/GTX4 comprise >90% of the total PSP toxin content on a molar basis. This toxin composition is consistent with that determined for *A. catenella* populations from the Pacific coast in the northern hemisphere. The characteristic toxin profile is also reflected in the shellfish, but with evidence of epimerization and metabolic transformations of C1 and C2 to GTX2 and GTX3, respectively. This work represents the first unequivocal identification and confirmation of such PSP toxin components from the Chilean coast.

Keywords: *Alexandrium catenella*; LC-FD; LC–MS/MS; PSP toxins; Toxic dinoflagellates

1. Introduction

The tetrahydropurine toxins associated with paralytic shellfish poisoning (PSP) are potent neurotoxins active on voltage-gated sodium channels of excitable cells (Kao and Walker, 1982). These toxins can be divided into three structural groups (Fig. 1) based on the nature of the side chain: carbamoyl (R4 = $-CONH_2$), *N*-sulfocarbamoyl (R4 = $-CONHSO_3^-$) and decarbamoyl (R4 = –H). This family of PSP toxins includes saxitoxin and more than 20 naturally occurring related compounds (Onodera et al., 1997; Kodama, 2000). These toxins are produced by marine dinoflagellates belonging to the genera *Alexandrium*, *Pyrodinium* and *Gymnodinium*, as well as by certain species of freshwater and brackish water cyanobacteria (Hall et al., 1990; Carmichael, 1994). Suspension-feeding shellfish can be vectors of such phytoplanktonic toxins via accumulation in their tissues, but these toxins can also move directly through pelagic food chains, affecting zooplankton, fish, birds and marine mammals (Mortensen, 1985).

In Chile the presence of PSP is endemic in the southern part of the ocean littoral. In these Austral

* Corresponding author. Tel.: +49 4831 2055; fax: +49 4831 2115.
E-mail address: Bernd.Krock@awi.de (B. Krock).

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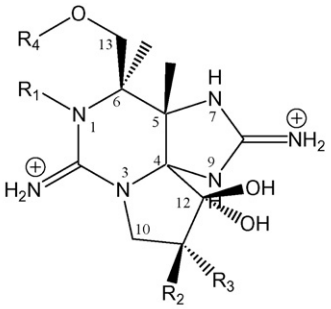

			Carbamoyl	N-Sulfocarbamoyl	Decarbamoyl
R1	R2	R3	R4: $CONH_2$	R4: $CONHSO_3^-$	R4: H
H	H	H	STX	B1	dcSTX
OH	H	H	NEO	B2	dcNEO
OH	H	OSO_3^-	GTX1	C3	dcGTX1
H	H	OSO_3^-	GTX2	C1	dcGTX2
H	OSO_3^-	H	GTX3	C2	dcGTX3
OH	OSO_3^-	H	GTX4	C4	dcGTX4

Fig. 1. Naturally occurring PSP toxins found among various marine dinoflagellates and toxin vector organisms, including carbamate, *N*-sulfocarbamoyl, and decarbamoyl derivatives. Saxitoxin = STX; neosaxitoxin = NEO; gonyautoxins 1,2,3,4 = GTX 1,2,3,4; B1 (=GTX5); B2 (=GTX6); dc = decarbamoyl toxins.

Regions, *Alexandrium catenella* has been reported as the main agent responsible for PSP toxin occurrence and shellfish contamination (Muñoz, 1985; Cassis et al., 2002; Lagos, 2003). *A. catenella* was first recorded in the Magellan strait in 1972 and since then its known range in Chilean waters has expanded from 55°55′S to 44°44′S. Furthermore, the seasonal frequency and dispersion also appears to be increasing (Guzmán et al., 2002). During the last three decades, several hundred people in Chile have suffered from PSP syndromes, and more than 25 humans have died after shellfish consumption. As a consequence, quarantines have frequently been imposed on shellfish collection, transportation and commercialization. Since 1991, PSP outbreaks have been recorded continuously in this geographical area. Most studies on PSP toxin occurrence and composition in Chile have been devoted to the analysis of these toxins in shellfish, such as the Chilean blue mussel *Mytilus chilensis* (Andrinolo et al., 2002; García et al., 2004), the striped mytilid (or Chilean ribbed mussel) *Aulacomya ater* (native name ''cholga'') (García et al., 2005), and two carnivorous gastropods, *Concholepas* (native name ''loco'') and *Argobuccinum ranelliformes* (native name ''caracol del sur'') (Compagnon et al., 1998). Some research has also been conducted on toxin kinetics and dynamics of PSP toxin components of *A. catenella* in mammalian subjects (Andrinolo et al., 2002). Nevertheless, the toxin profile of the causative organisms in Chile has not been heretofore clearly identified and confirmed by advanced analytical methods.

This article reports the first confirmatory analysis of a Chilean strain of *A. catenella* simultaneously carried out by liquid chromatography with post-column derivatization followed by fluorescence detection (LC-FD) and with tandem mass spectrometry (LC–MS/MS). These data are compared with PSP toxin profiles reported from mussels of the same temporal and