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Toxin variability in cultured and natural populations of *Alexandrium tamarense* from southern South America – Evidences of diversity and environmental regulation

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ABSTRACT

Cell content and composition of paralytic shellfish toxins of 10 cultured strains and 6 natural populations of *Alexandrium tamarense* from the Argentine sea, were analyzed. These data were compiled with previously published data into a comprehensive view of the toxin composition of the complex *A. tamarense*/*Alexandrium catenella* from southern South America.

The N-sulfocarbamoyl derivatives C1,2 were predominant in almost all the cultured strains. The second major derivatives were GTX1,4, although the GTX1,4/C1,2 ratio varied largely. Some strains contain relatively high amounts of GTX2,3 (up to 29%) and/or neoSTX (up to 24%). In all strains STX was a minor component (0–4.4%) whereas GTX5 was present only in *Alexandrium catenella* isolates. Similarity analysis based upon toxin profiles showed that cultured strains from Argentine, Brazil, Chile and Uruguay clustered together. However, whereas some strains from the same geographic area exhibited different toxin profiles, and consistently fell out in separate subgroups, strains from Chile are grouped in a unique subgroup. In contrast to cultured strains, C1,2 were minor components among field populations. The highly toxic GTX1,4 were predominant in all spring field populations (69.1–93.6%). Moreover, their toxin cell content (163.9–261.4 fmol cell^{-1}) and toxicity (68.2–93.0 pg STX equiv. cell^{-1}) were several times higher that showed by the cultured strains. Field populations are more closely related to one another than to the cultured strains. However a less toxic and morphologically distinctive autumn population, contained GTX2,3 as the quasi unique (88.5%) toxin derivative clustered separately. Variability in toxin content and composition of *A. tamarense* field populations were well correlated with *in situ* temperature and nitrate concentration. Whereas toxin cell content and GTX1,4 (mol %) increased following saturation functions, GTX2,3 (mol %) decrease exponentially with the increase of the *in situ* nitrate concentration.

1. Introduction

Alexandrium is an important dinoflagellate genus that comprises many toxic species whose distribution area is spreading into various parts of the world recently. Most of toxic *Alexandrium* dinoflagellates produce potent neurotoxins called saxitoxin (STX) analogues (about 24 known STX derivatives that differ in structure and toxicity) or paralytic shellfish toxins (PSTs), which accumulate and are metabolized in shellfish (Etheridge, 2010 and references therein).

In both margins of South America, seasonal blooms of several toxic species of the genus *Alexandrium* (*Alexandrium tamarense*; *Alexandrium catenella*; *Alexandrium minutum*

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and *Alexandrium tamiyavanichii*) have been well documented (Carreto et al., 1986, 2002; Guzmán et al., 2002; Odebrecht et al., 2002; Menezes et al., 2007, 2008). However, thus far, the increased reports of human health and economic impacts had been caused by the increased in intensity and geographic spread of *A. catenella* and *A. tamarense* blooms (Carreto et al., 2002; Guzman et al., 2002; Persich et al., 2006).

A. tamarense and *A. catenella* were defined originally based on constant morphological characteristics (Fukuyo, 1985; Balech, 1995). Among these, the presence of a ventral pore on the first apical plate and chain forming ability was thought to be the most reliable characters to distinguish *A. tamarense* from *A. catenella* (Fukuyo, 1985). However, Gayoso and Fulco (2006) reported that the presence of the ventral pore and the chain forming ability in *A. tamarense* cells collected from field populations of Golfo Nuevo (Argentina) as well as between clones, even in the same clone grown under different temperature and irradiance, varies considerably. Moreover, recent results showed that populations on the east coast of South America and Chile have identical D1–D2 LSU rRNA gene sequences, which may demonstrate that the eastern populations were originated recently from Chile (Persich et al., 2006; Lilly et al., 2007). Therefore, the observed characteristic distribution of *A. tamarense* and *A. catenella* morphospecies at the east and west coast of South America, respectively (Carreto et al., 2002; Guzmán et al., 2002), may be the result of the adaptive morphological plasticity of *Alexandrium* populations (Gayoso and Fulco, 2006).

Toxin composition in nutrient replete cultured strains has been considered a stable feature a fixed genetic trait that can be used to distinguish strains or species, as a biochemical fingerprint (Cembella, 1998). Recently, however, significant changes in toxin composition have been reported in cells exposed to different stresses (Etheridge and Roesler, 2005; Poulton et al., 2005). Although a comprehensive view of the toxin composition of the complex *A. tamarense/A. catenella* from southern South America is lacking, preliminary results showed that under nutrient replete conditions, the low potency N-sulfocarbamoil toxins C1,2 were predominant in almost all strains analyzed (Carreto et al., 1996, 2001; Méndez et al., 2001; Persich et al., 2006). One exception was a rare highly toxic strain isolated from Los Patos Lagoon (Brazil) (Persich et al., 2006). In contrast to cultures, toxin composition of South American *Alexandrium* field populations has not been examined in detail. Among cultured strains isolates from Argentine (Carreto et al., 1996, 2001; Frangópulos et al., 2004), Uruguay (Méndez et al., 2001) and Brazil (Persich et al., 2006) cell toxicity ranged from 2,3 to 65,6 pg STX equiv. cell^{-1}. Therefore, the observed differences in toxicity and bloom dynamic among regions (Carreto et al., 1998) may be due to differential geographic distribution of *Alexandrium* subpopulations. On the other hand, environmental parameters such as irradiance, temperature, salinity or inorganic nutrients have been shown to affect toxin content and composition for several different *Alexandrium* strains (Etheridge and Roesler, 2005). Therefore, another potential reason for differences in regional toxicity could be due to isolate-specific responses to environmental conditions.

In this study cell content and composition of paralytic shellfish toxins of 10 cultured strains and 6 natural populations of *A. tamarense* from the Argentine sea, were analyzed. These data are compiled with previously published data including strains from Brazil, Uruguay, Argentine and Chile into a comprehensive view of the toxin composition of cultured and natural populations of *A. tamarense/A. catenella* complex from southern South America. We also explore if the variability of toxin content and composition of *A. tamarense* field populations were correlated with the prevailing environmental conditions.

2. Material and methods

2.1. Strains and cultured conditions

Clonal cultures of *A. tamarense* were established from vegetative cells from coastal waters of Mar del Plata, Nuevo Gulf, San José Gulf, and from the Patagonian Shelf waters off Peninsula de Valdes (Table 1). The clones were isolated by means of a micro-capillary pipette and maintained as unialgal stock cultures under standardized growth in f/2 medium without silicate (Guillard and Ryther, 1962) at 15 °C under a 12–12 h light–dark cycle and ca 100–200 μmol photon m^{-2} s^{-1} provided by cool white fluorescent tubes. Cultures were harvested for toxin analysis during the mid-exponential growth phase. Strain numbers and isolation sources for *A. tamarense* cultures analyzed in this study are showed in Table 1.

2.2. Sampling of field populations

2.2.1. Mar del Plata coast

Sampling was done during one-day cruises to the permanent monitoring station off Mar del Plata (EPEA 38°28′S 57°41′W), at monthly intervals, on board the research vessel "Capitán Cánepa". During the cruises, temperature and salinity profiles were determined by means of a Seabird SBE1901 CTD. Water samples for the determination of nutrients, chlorophyll-a concentration, pigments concentrations and identification of phytoplankton composition were taken from surface (0m depth) with a bucket and from different depths using Niskin bottles. Vertical hauls were used to obtain qualitative phytoplankton samples. Mussel samples (*Mytilus edulis*) were taken from the benthic community at the EPEA station (45 m depth) using a dredge. Seawater samples for nutrient determination were kept at −20 °C until analysis at the laboratory. Since low toxin concentrations measured at the beginning and end of the bloom make it difficult to interpret changes in toxin concentration that might have occurred as the bloom declined, surface phytoplankton samples for toxin analysis were collected during the largest peak of *A. tamarense* blooms occurred during October 1996, April 2000 and October 2000 (Table 2). During October 1996 the sample was collected using 20-μm plankton net. The collected material was then back-washed onto a 20 μm sieve and re-suspended into a conical centrifuge tube. The suspension was filtered onto a Whatman glass fiber filters and then frozen at −21 °C until analysis. During April and