PSP TOXIN PRODUCTION OF URUGUYAN ISOLATES OF *GYMNODINIUM CATENATUM* AND *ALEXANDRIUM TAMARENSE*

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ABSTRACT

It has been well documented by the National Monitoring Programme on HAB and Mussel Toxicity in Uruguay that *Alexandrium tamarense* was present during paralytic shellfish poisoning (PSP) episodes along the coast of Uruguay in the late winter/early spring of 1991, '92, '93, '95 and '96 while *Gymnodinium catenatum* was present during PSP events in the late summer/early fall of 1992, '93, '94, '96 and '98. Cultures of these two dinoflagellates, established from the mouth of the Rio de la Plata, have been analyzed for saxitoxin and its derivatives by high performance liquid chromatography (HPLC) for the first time. These toxin profiles have been compared to shellfish extracts taken during four different toxic booms. There is a strong similarity between the toxin profiles of the shellfish collected in September 1991 and August 1993 and cultured *Alexandrium* extracts whereas the best agreement between shellfish extracts, from March of 1993 and 1994 is with cultured *G. catenatum* extracts. These results demonstrate for the unequivocally that PSP in Uruguayan shellfish can arise from two different dinoflagellate species in two different seasons. This has clear implications with respect to the design and operation of an effective monitoring program.

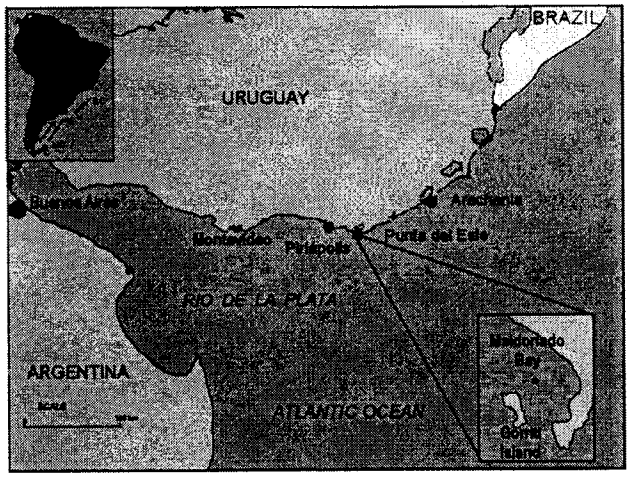

Fig.1. Map of the study area showing sample sites. Shellfish and phytoplankton samples collected at Piriápolis, Punta del Este and Arachania; sediment samples collected at Maldonado Bay (lower insert). The Subtropical Convergence Zone is seen in the upper insert.

INTRODUCTION

The coastal area influenced by the nutrient-rich waters of the Rio de la Plata (Fig. 1) is a very productive habitat [1]. Since the 1980's, impacts caused by frequent harmful algal blooms (HABS) have had a significant effect on the local economy due to decreased fishery and tourist revenues and the public's negative perception of seafood safety [2,3,4]. In 1980 the first toxic outbreak was recorded [5,6] and in the years since then, recurring episodes of PSP in molluscs at times of *Alexandrium tamarense* and *Gymnodinium catenatum* blooms have been recorded. There have, however, not been any detailed toxin analyses from these events.

MATERIAL AND METHODS

Study area

The Rio de la Plata (Fig. 1) is the second largest South American basin, draining an area of approximately 3,170,000 km^2 that extends into five countries. Salinity and temperature of this water body fluctuate dramatically through the year with salinity ranging between 5 and 32 g/L^{-1}, and the annual temperature between 8 and 25 °C. [7]. Seasonal winds, in combination with currents within the Subtropical Convergence Zone (Fig. 1), have a major influence on the Rio and its phytoplankton population dynamics [8,9].

Cultures

Shortly after blooms of *A. tamarense* and *G. catenatum*, sediment samples were collected from the Bay of Maldonado near Punta del Este (Fig. 1). Two clonal cultures of *G. catenatum*, GCURD5 and GCURF11, and three clonal cultures of *A. tamarense*, ATUR01, ATUR01B and ATUR02, were established from germinated cysts isolated from these sediments. Upon reaching mid-exponential growth, duplicate cultures were extracted for PSP toxins with both mild, 0.5 M acetic acid (HOAc) to preserve to original cellular toxin composition, and 0.1 M hydrochloric acid (HCl), heated to convert the N-sulfocarbamoyl toxins to their more potent carbamate derivatives.

Shellfish

Blue mussels, *Mytilus edulis*, were harvested during blooms of *A. tamarense* from the Punta del Este station in the Bay of Maldonado on 4 September 1991 (*Mytilus* 1) and 26 August 1993 (*Mytilus* 3). *Donax hanleyanus*,

a commensal clam, and *M. edulis* were each collected from the Arachania and Piriapois stations on 2 March 1994, (*Donax* 4) and 24 March 1993 (*Mytilus* 2) respectively during blooms of *G. catenatum* (Fig. 1). All shellfish samples were processed according to the standard method of the Association of Official Analytical Chemists (AOAC) [10]. Because there was incomplete hydrolysis of these extracts during the original processing, a subsample was mixed 1:1 with 0.2 N HCl and rehydrolyzed.

All samples were analyzed by HPLC [11] for the saxitoxins using a modified three-step isocratic elution method [12]. External standard solutions, kindly provided by Y. Oshima (Tohoko University, Sendi, Japan), were run prior to the commencement of sample analysis and after every fourth sample. The terms GTX1,4, GTX2,3, C1,2 and C3,4 are used to represent the pooled concentrations of two toxins so as to account for possible epimerization. Toxicities (in STX equivalent cell^{-1}) were calculated from molar composition data using individual potencies [11].

RESULTS AND DISCUSSION

Bloom details

Alexandrium tamarense favors the cooler waters (8-18 °C) of the late winter, early spring season with maximum cell densities of over 30,000 cells L^{-1} witnessed in each of the two blooms reported here. Bloom initiation and subsequent enhancement may result from transport of the cells from southern, offshore waters coupled with local *in situ* growth. These same phenomena may also give rise to spring blooms of *A. tamarense* along the coast of Argentina [13, 14].

Gymnodinium catenatum has reached cell densities of over 60,000 cells L^{-1} when warmer water temperatures (19-24 °C) prevail during the late summer and early fall months of the southern hemisphere which are consistent with distributions of this species along the Argentine coast [15, 16]. However, blooms of *G. catenatum* in the Rio de la Plata are considered to be more localized events, as seedbeds of *G. catenatum* cysts, which contain more than 400 cells cc^{-1} sediment, have been discovered in the nearshore area (17, 18).

Toxin content and composition: cultures

HPLC analysis of acetic acid extracts of the two dinoflagellate species reveals that the two *G. catenatum* cultures, on average, had the greatest amount of toxin per cell on a molar basis, (193 vs 70 fmol/cell^{-1}) but when the potency of these same extracts is considered, the overwhelming proportion, up to 96 mole percent of the N-sulfocarbamoyl derivative toxins C1-4, GTX5 and GTX6 which is typical for *G. catenatum* strains worldwide [19, 20]. The more potent carbamate toxins such as GTX1, 4 and GTX2, 3 that were found in the *A. tamarense* cultures are only detected in very low levels in the *G. catenatum* extracts (Fig. 2a). While the *Alexandrium* cultures had significant levels of the gonyautoxins, ranging from 24 to as much as 47 mole

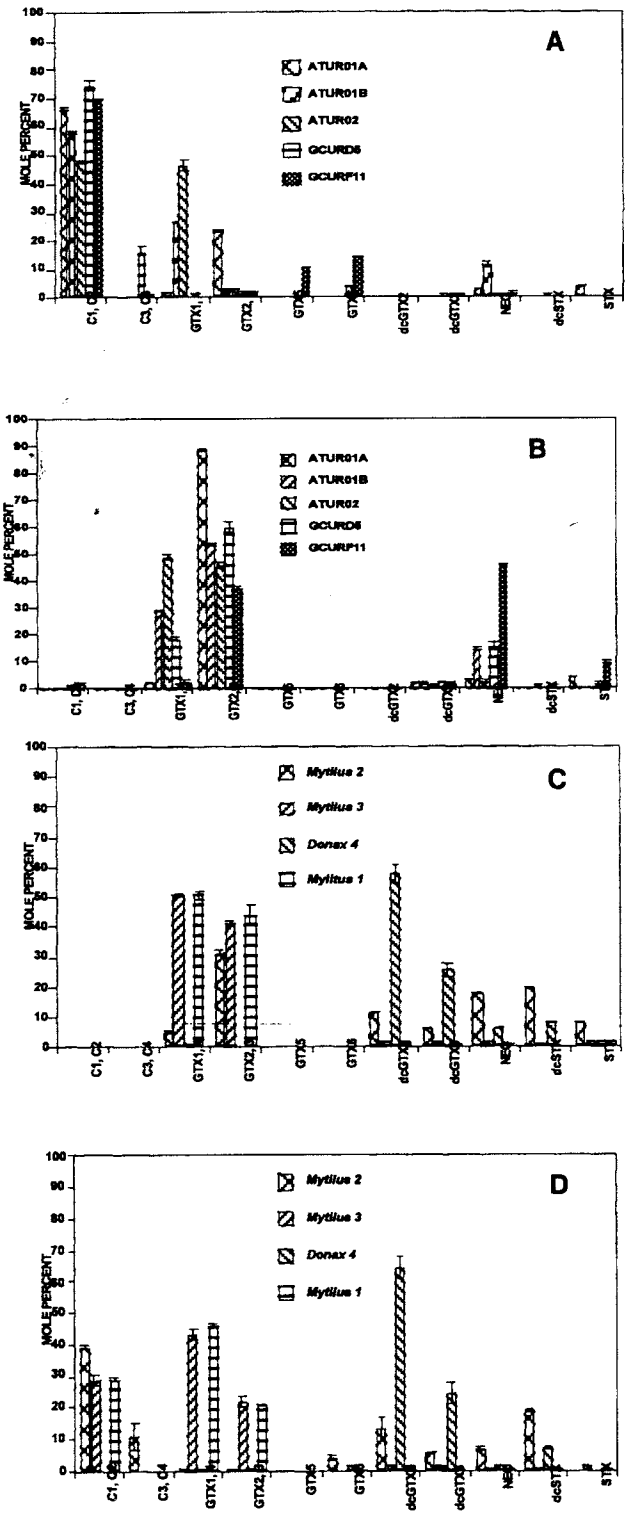

Fig. 2. Mole percent toxin composition of dinoflagellate and shellfish extracts. (A) Culture acetic acid extracts; (B) Culture hydrochloric acid extracts; (C) Original shellfish extracts; (D) Rehydrolyzed shellfish extracts.

Alexandrium isolates are seen to be more toxic (15292 vs. 12200 fgSTX equiv. cell^{-1}). The lower toxicity values associated with higher molar concentrations of toxin in the *G. catenatum* cultures result from an percent, they were still dominated by the low potency C1 and C2 toxins, 49–67% (Fig. 2a). Lesser amounts of NEO and STX were contained in two of the three *Alexandrium* strains (<5%) however, ATUR01B was found to have 11% NEO (Fig. 2a).

The toxicity of all these cultures changes if they are subjected to hot acid treatment that converts the less potent N-sulfocarbamoyl derivatives to their highly toxic analogues. Following these preparations, the *G. catenatum* cultures still contained an elevated molar concentration of toxin, but were now much more potent on a cellular basis than the *A. tamarense* cultures, 76,660 vs. 21,221 fgSTX equiv. cell^{-1}. This is an increase in *G. catenatum* toxicity of over 600% from the original acetic acid extracts resulting from the creation of high amounts of GTX1,4 and GTX2,3 and NEO following hydrolysis (Fig. 2b). Gonyautoxins 2,3 and 1,4 dominate the *A. tamarense* hot acid extracts with combined values ranging from 83 to 95 mole percent (Fig. 2b).

Toxin composition: shellfish

Prior to rehydrolysis, all of the shellfish extracts, with the exception of sample *Donax* 4, harbored significant levels of the N-sulfocarbamoyl derivative toxins, up to a maximum of 53%, presumably due to incomplete hydrolysis of the sample during the initial processing (Fig 2c). Sample *Donax* 4, collected during a *G. catenatum* bloom, was unique in that it was chiefly composed of decarbamoyl toxins, primarily dcGTX2, prior to, and after rehydrolysis.

The other shellfish sample taken during a *G. catenatum* episode, *Mytilus* 2, was dominated by the low potency N-sulfocarbamoyl derivatives (53%) while also having significant amounts of decarbamoyl toxins GTX2, GTX3 and STX (Fig. 2c). As would be expected, GTX 2,3 and 1,4 as well as NEO mole percents all increased with rehydrolysis of this sample (Fig. 2d).

The two samples, *Mytilus* 1 and *Mytilus* 3, harvested during the *Alexandrium* blooms, had strikingly similar toxin profiles. In the original extract, toxins C1,2, GTX1,4 and GTX2,3 were found to compose over 94 mole percent of the total toxin profile with 29% arising from C1,2 (Fig 2c). After rehydrolysis, toxins C1,2 were converted to GTX2,3 with its concentration increasing from about 21% to 42% of the total molar toxin composition while the percentage of toxins GTX1,4 increased marginally to about 50% of that total (Fig. 2d).

Toxin composition comparisons, cultures vs. shellfish

The fact that the shellfish samples were not completely hydrolyzed by the AOAC extraction procedure is rather disconcerting from a public health and regulatory standpoint, but it proved to be an asset in comparing the toxin profiles of the shellfish and the phytoplankton culture extracts. While the agreement between the toxin profiles of the cultures and the shellfish is not perfect, there is sufficient similarity in the case of shellfish samples *Mytilus* 1, *Mytilus* 2 and *Mytilus* 3 and the cultured dinoflagellate extracts to establish correlations. These are supported by the phytoplankton observations made when the shellfish samples were collected.

The original extracts of shellfish samples *Mytilus* 1 and *Mytilus* 3 had profiles dominated by toxins C1,2, GTX1,4 and GTX2,3. Toxin profiles of *A. tamarense* cultures ATUR01A, ATUR01B and ATUR02 were also dominated by the same epimer groups although the percent composition of each epimer pair in the shellfish and cultured samples were somewhat different (Fig. 2a,c). This discrepancy in mole percent toxin composition can be explained by compositional shifts resulting from metabolic processes (bioconversion) within the shellfish [21, 22, 23, 24] and by the conversion of the low potency N-sulfocarbamoyl derivative toxins to their more potent carbamate forms by hot acid treatment [25]. While there is no way of knowing what the original unhydrolyzed toxin profiles of the dinoflagellates which rendered these mussels toxic was, these data suggest that they are most similar in composition to the cultured *A. tamarense* and not *G. catenatum* from those waters.

The two shellfish samples collected during *G. catenatum* blooms are quite different from one another and only one, *Mytilus* 2, is similar in composition to either of the two *G. catenatum* cultures based on the high proportions of toxins C1,2, C3,4, and GTX6. The toxin profile of shellfish sample *Donax* 4 was dominated by decarbamoyl toxins GTX2, GTX3 and STX and the only indication that *G. catenatum* may have rendered this sample toxic are the low levels of GTX6 (<2 mole percent), that are seen only in the *G. catenatum* extracts, and are found in this sample. These low concentrations of GTX6 are validated by the rehydrolysis data, which reveal an increase in NEO concentrations that coincides with the concentration of GTX6 measured in the original extract. While specific mechanisms for the transformation of toxins in *Donax* have not been investigated, one can postulate that *in vivo* enzymatic decarbamoylation of toxins C1,2 or GTX2,3, described in the other bivalve species occurred resulting in the high percentage of decarbamoyl derivatives [26, 27, 28]

SUMMARY

This paper describes for the first time toxin profiles of two causative organisms responsible for PSP events in Uruguay and links these profiles to those of toxic shellfish harvested during blooms of the two dinoflagellates.

The blooms of *A. tamarense* appear to be restricted to the late winter/early spring seasons and are initiated by the cold current transport of cell populations from southern offshore waters. These cells have toxin profiles rich in the high potency gonyautoxins but contain less

toxin per cell on a molar basis than the *G. catenatum* isolates.

Gymnodinium catenatum is dominated by low potency N-sulfocarbamoyl toxins that are converted to their highly toxic carbamate derivatives by hot acid hydrolysis. Blooms of this species occur during the late summer/early fall season when water temperatures are significantly warmer than those measured during *A. tamarense* blooms. High numbers of *G. catenatum* cysts within the Rio de la Plata suggest that these blooms maybe a result of localized cyst germination events.

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