Genetic Variability and Toxin Profile of *Alexandrium tamarense* (Lebour) Balech from Southern Brazil

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Abstract

The distribution of the toxic dinoflagellate *Alexandrium tamarense* (Lebour) Balech has apparently expanded to or within the Southern Hemisphere during the last two decades. Toxic blooms of *A. tamarense* have been recorded in Argentinean coastal waters since 1980; however, the first documented bloom in southern Brazil was in 1996. In this study, 13 strains of *A. tamarense* from southern Brazil were isolated and kept in culture. Phylogenetic analysis using RFLP (Restriction Fragment Length Polymorphism) and DNA sequence of the D1-D2 region of large subunit ribosomal DNA (rDNA) places the Brazilian strains firmly within the North American clade, but does not assign them to any existing subclade. The cultures were also analyzed for saxitoxin and its derivatives by high performance liquid chromatography (HPLC). The main saxitoxin groups found were the low toxicity N-sulfocarbamoyl group C1+2 (30–84%), followed by the high potency carbamate toxins, gonyautoxins 1+4 (6.6–55%), gonyautoxins 2+3 (0.3–29%), neosaxitoxin (1.4–24%) and saxitoxin (0–4.4%). Toxin levels were variable (7,000 to 66,000 fg STX cell^{-1}), with the higher range falling among the most toxic values recorded for cultures of *A. tamarense,* indicating the significant risk for shellfish contamination and human intoxication during blooms of this species along the southern Brazilian coast. Possible dispersal hypotheses such as ballast water transport and natural mechanisms are also discussed.

Introduction

In South America, the range of *A. tamarense* appears recently to have spread around the southern tip and northward along the east coast. The earliest South American PSP outbreak was recorded in 1886 in Chile, associated with *A. catenella* (Sengers, 1908); the next outbreak was reported in 1972 (Guzmán *et al.,* 1975). In the South Atlantic, the first toxic outbreak of *A. tamarense* occurred in Argentina in 1980 (Carreto *et al.,* 1985); since then, this species has been periodically detected along that coast. In Uruguay, the first PSP outbreak also was recorded in 1980, but conclusive identification of *A. tamarense* as the causative species was not possible until the second toxic outbreak in 1991 (Brazeiro *et al.,* 1997). *A. tamarense* was first documented in southern Brazil in 1996, concomitant with a bloom in Uruguay (Odebrecht *et al.,* 1997). We hypothesized that the southern Brazilian *A. tamarense* populations were introduced by natural currents from Uruguay. This study represents the first attempt to isolate, determine the toxin composition profile, and uncover the origin of the *A. tamarense* population from southern Brazil, using both genetic analysis and historic accounts.

Materials and Methods

Origin of the Strains Sediment samples were collected from March to November, 1997 in Praia do Cassino (32°04′ to 32°30′S; 51°49′ to 52°10′W). Sediment cyst concentrations were estimated by counts in Sedgwick-Rafter chambers, following the staining method in Yamaguchi *et al.* (1995). Twelve clonal strains were developed by cyst isolation, germination and re-isolation of individual motile cells. Strain ATBRC6 was established from a vegetative cell isolated from the water column in August 1997. Cultures were kept in f/2 medium (-Si) at 20°C under a 14:10 h light:dark cycle and ca. 350 μE m^{-2} s^{-1} and were harvested for all analyses in mid-exponential growth phase.

Toxin Analysis Toxin analyses were carried out in triplicate using a modification of the Oshima (1995) post-column derivatization HPLC method (Anderson *et al.,* 1994). Unknowns were identified and quantified based on standard reference material provided by Y. Oshima, and the toxicity values, in saxitoxin equivalents, were converted from the molar concentrations using the factors found in Oshima (1995).

Genetic Analysis RFLP assays of LSU rDNA were performed using D1R and D2C primers and the enzymes *Nsp* 1, *Mse* 1, and *Apa* L1 according to Scholin and Anderson (1996). RFLP patterns from our strains were compared to published data (Scholin and Anderson 1996) and unpublished data generated by one of the authors (Lilly) for strains from Chile, Argentina and Uruguay. The entire D1-D2 region was sequenced for four strains, ATBR2c, 2d, 2e and 2f, and aligned with existing *Alexandrium* sequences from Scholin *et al.* (1994) and the sequence of a Chilean isolate (Lilly, unpublished data). The most appropriate substitution model was determined using ModelTest (Posada and Crandall, 1998). Using Paup version 4.0b8 (Swofford, 2002), one thousand replicates of maximum parsimony analysis were run to generate starting trees for maximum likelihood analysis. One hundred replicates of fast-step bootstrap were run using maximum likelihood.

Results

Cyst Distribution Cyst concentrations varied between undetectable values and 179 cysts cm^{-3} at the northern station (Fig. 1). These concentrations are substantially lower

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

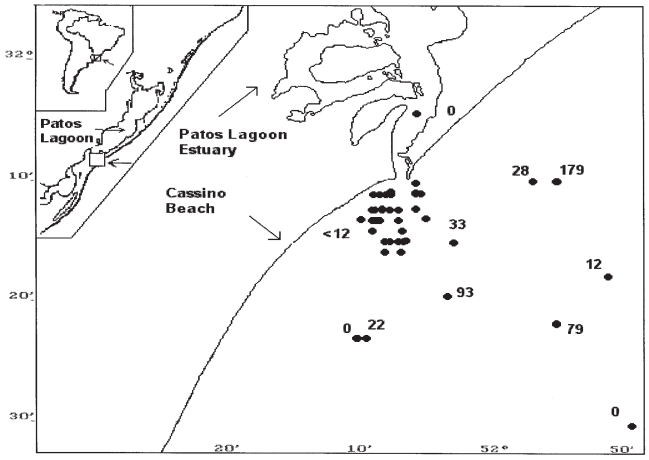

Figure 1 Sampling area with *A. tamarense* cyst concentrations (cysts cm^{-3}).

than the concentrations of cysts found in Argentina, which are up to 9,000 cysts cm^{-3} (Orozco and Carreto, 1987).

Toxin Analysis Total toxin concentration ranged from 42 to 199 fmol $cell^{-1}$ and toxicity from 7,000 to 65,900 fg STX eq $cell^{-1}$ in most of the strains, up to 78.4% of the total toxin (Fig. 2). The only exception was strain ATBRC6, whose higher toxicity value was due to the predominance of the potent GTX4.

Genetic Analysis RFLP analyses were inconclusive due to possible contamination issues. They indicated that the Western North American and the Eastern North American ribotypes might both be represented. Strains from Chile, Argentina and Uruguay display the Western North American ribotype (Lilly *et al.,* 2002). Sequence analyses were run on newly isolated material from four isolates that displayed the full range of patterns to clarify this issue. The sequences were identical to one another. The Brazilian sequences had several base changes in common with each of the Western, Eastern and Alternate North American ribotypes published by Scholin *et al.* (1994), but did not possess all of the base changes common to any of the groups. There was only one sequence difference with a Chilean strain. Phylogenetic analysis places the Brazilian strains firmly within the North American clade, but they do not fit within any existing subclade (Fig. 3).

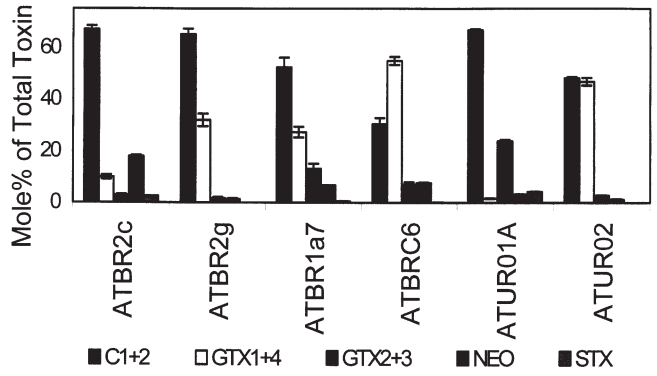

Figure 2 Toxin composition of representative Brazilian (ATBR) and Uruguayan (ATUR) strains.

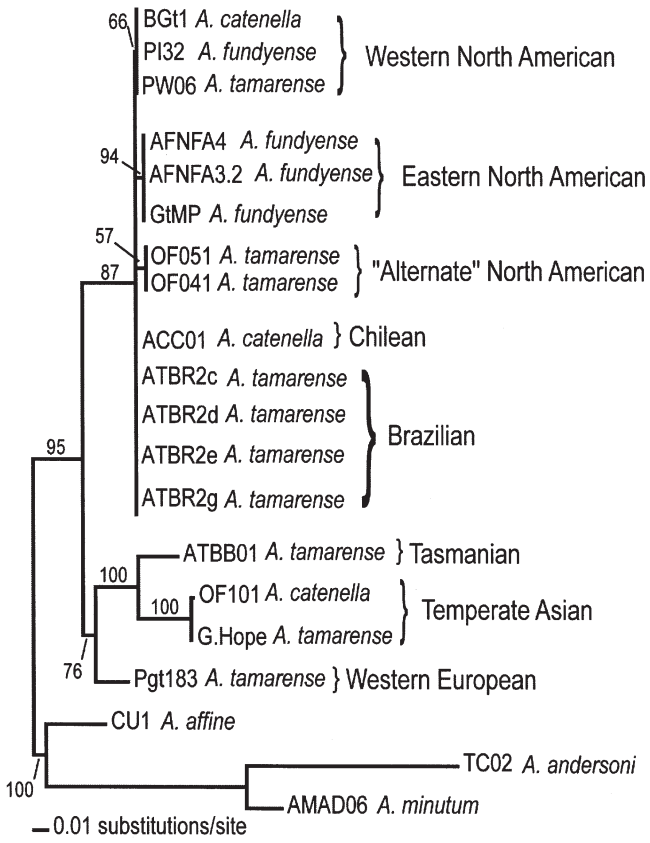

Figure 3 Maximum Likelihood tree showing the phylogenetic placement of the Brazilian strains within the *tamarensis* complex. Numbers represent 100 replicates of ML bootstrap.

Discussion

The occurrence of the North American pattern in *Alexandrium tamarense* from Brazil indicates a closer relationship with strains from the Northern Hemisphere than with strains from Australia or South Africa. Natural transport between North and South America in modern times is unlikely, since there are strong barriers, including temperature. One possible explanation could be that *Alexandrium* were transported in the ballast water of cargo vessels from the North American or Japanese coasts, where the North American ribotypes have also been detected (Scholin *et al.,* 1994). The other possibility is that the *Alexandrium* populations in South America were established much earlier during a period of cooler global ocean temperatures when natural transport across the hemispheres was possible, such as the last ice age. The DNA sequence data support this hypothesis. The South American LSU rDNA sequences are intermediate between the Asian, Western North American and Eastern North American populations of the North American ribotype, indicating that the populations have

been separated long enough for evolution to have occurred.

By either means, it is most likely that *A. tamarensis* complex cells first arrived in South America along the western coast and then spread to eastern South America via current systems. This is supported by the historical precedence of PSP in Chile, but not in other South American countries (Sengers, 1908, Guzmán *et al.,* 1975). PSP outbreaks subsequently occurred in Argentina, Uruguay and most recently, southern Brazil. Experimental and field observations also support this. First, there is the genetic pattern shared by the Chilean, Argentinean, Uruguayan and Brazilian strains, as shown with RFLP and sequence data (Lilly *et al.,* 2002). There is also a correlation of *Alexandrium* populations and presence of frontal systems in both Argentina and Uruguay (Carreto *et al.,* 1986 and Brazeiro *et al.,* 1997). Lastly, the high similarity between Brazilian and Uruguayan *Alexandrium* toxin profiles (present study and Mendéz *et al.,* 2001, respectively, Fig. 2) indicates a close relationship between these populations, as strains from each region have a high percentage of C1+2 toxins (30–80%), and lesser, but still an elevated mole percentage of the following toxins: GTX1+4 (2–55%), GTX2+3 (2–29%) and NEO (1–24%). Very low amounts of dcGTX3 and dcSTX and STX were seen in some of the isolates from each country as well.

Cyst concentrations in Brazilian sediments are lower than those in Argentina, but further study is needed to determine the reasons for this. Cultures derived from these cysts and vegetative cells can be highly toxic, with the higher range falling among the most toxic values recorded for *A. tamarense* cultures. In the future, we can expect additional toxic outbreaks from offshore cyst germination, or from new populations that are transported via Uruguayan coastal currents.

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