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# Growth and toxin profile of *Ostreopsis* cf. *ovata* (Dinophyta) from Rio de Janeiro, Brazil

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ABSTRACT

Since 1998, blooms of the epi-benthic dinoflagellate, *Ostreopsis* cf. *ovata* Fukuyo have been reported in the Arraial do Cabo area, Rio de Janeiro, Brazil. This dinoflagellate can produce one of the most lethal marine toxins, palytoxin (PLTX) and its analogues. In the study reported herein, the growth rate, cellular dimensions and toxin profile of two *O.* cf. *ovata* strains isolated from Armação dos Búzios, Rio de Janeiro were investigated. Molecular analysis (5.8S rDNA gene and ITS regions) of one strain was also performed. Respectively, strains LCA-E7 and LCA-B7 showed growth rates of 0.15 and 0.10 div $day^{-1}$, and cell dimensions of the strains and field population were similar to those found for *O. ovata* from the Mediterranean Sea. Ribosomal DNA genetic sequences obtained from the nuclear region (ITSs and 5.8S) of strain LCA-E7 confirmed that this is *O.* cf. *ovata* and grouped this isolate in a robust clade with isolates from Brazil and the Mediterranean. The production of putative (p)PLTX and ovatoxin (OVTX) was assessed using ouabain inhibited hemolytic assays. Extracts of the two strains caused hemolysis and was positively related to *O.* cf. *ovata* cell numbers. Analysis by liquid chromatography tandem mass spectrometry identified OVTX-a, -b, -c, -d and -e. Estimated concentrations varied between 20 and 171 pg[OVTX-a] $cell^{-1}$, 23 and 205 pg[OVTX-b] $cell^{-1}$, 3 and 37 pg[OVTX-c] $cell^{-1}$ and 3 and 80 pg[OVTX-d + e] $cell^{-1}$. OVTX-a and -b were the major components of the toxin profile produced by strains LCA-E7 and LCA-B7 and represented between 45 and 95% of the total OVTX + pPLTX content of these cells. Concentrations of OVTXs produced by the Brazilian strains were higher than those found in an *O.* cf. *ovata* Adriatic isolate, although in the same magnitude.



## 1. Introduction

*Ostreopsis ovata* Fukuyo (family Ostreopsidaceae) is an epiphytic-benthic dinoflagellate that is found to occur within tropical and temperate latitudes (Rhodes, 2011). It is generally associated with macroalgae, rocks and sediments and frequently co-exists with other epi-benthic dinoflagellate species such as *Gambierdiscus*, *Coolia*, *Prorocentrum* and *Amphidinium*. Of the species belonging to this genus, *O. ovata* is possibly the most conspicuous. The organism can synthesize a palytoxin-like molecule and similarly structured analytes such as ovatoxins-a, -b, -c, -d & -e (Ciminiello et al., 2010). Palytoxin (PLTX) has been described as one of the most potent toxins known (Moore and Scheuer, 1971). It has been the cause of human poisoning through the consumption of contaminated seafood such as crabs (Alcala et al., 1988), parrotfish (Noguchi et al., 1988) and clupeotoxic sardine (Onuma et al., 1999).

Partly due to intensive field sampling and a greater ease of species identification (Rhodes, 2011), the understanding of the global distribution of *O. ovata* has expanded in recent years. However, there has also been a measurable increase in *Ostreopsis* blooms in areas that are regularly monitored, such as New Zealand and the Mediterranean Sea (Rhodes, 2011). Moreover, harmful effects of these unusual proliferations, including intoxication of sea goers in the Mediterranean (Ciminiello et al., 2006) and sea urchins poisoning in Brazil (Granéli et al., 2002; Ferreira, 2006) and New Zealand (Shears and Ross, 2009), as well as impacts to the benthic fauna (Sansoni et al., 2003) have only been reported in the last decade.

Massive blooms of *O. ovata* have occurred in the Mediterranean Sea since summer 1998. Following its presence along the Ligurian

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coast in summer 2005 (Genoa, Italy), hundreds of people required medical attention after exposure to marine aerosols during recreational activities (Ciminiello et al., 2006). Symptoms of human intoxication included fever, bronchoconstriction with mild dyspnea, wheezing, conjunctivitis and skin irritation. Recently, the putative (p)PLTX was reported for the first time in shellfish from the Aegean Sea (Greece) and coincided with the presence of *Ostreopsis* spp. (Aligizaki et al., 2008). The mode of action due to palytoxin is by the compound binding to the $Na^+/K^+$ ATPase pump in a configuration that permits the ions to flow in both directions and thus, altering cell permeability to monovalent cations (Artigas and Gadsby, 2003).

In Brazil, *Ostreopsis* sp. was first identified in the São Sebastião Channel, São Paulo (23°48′S; 45°25′W). Extracts from an isolate showed hemolytic activity and an inhibitory effect on the development of sea urchin (*Echinometra lucunter*) eggs (Naves and Freitas, 2001). In 2004 *O.* cf. *ovata* has also been observed associated to a *Trichodesmium* bloom in the Arvoredo Island, Santa Catarina (27°35′S, 48°34′W), south of Brazil (Silva et al., 2006). Extensive blooms of *O.* cf. *ovata* have been recorded in Arraial do Cabo, Rio de Janeiro (22°58′S, 42°1′W), southeast Brazil, in summer 1999 and 2002. Reported as ostreocins, analogues of PLTX were identified from the *O.* cf. *ovata* bloom in 2002 (Granéli et al., 2002) and these blooms were reported by Granéli et al. (2002) and Ferreira (2006) to decimate the local sea urchin population. Blooms of *O.* cf. *ovata* were also observed in June and December 2006, and again, in April and September 2007 in Arraial do Cabo and Armação dos Búzios, Rio de Janeiro (Nascimento et al., 2008, 2010). Although effects on local sea urchins were not significant, there was evidence of a loss of spines in some periods of high cell densities. In the Brazilian northeast, *O.* cf. *ovata* was registered in 2006 and 2007 in Pernambuco (8°24′S; 35°4′W; Nascimento, 2006 and data not published) and in Bahia (16°24′S; 39°02′W; Proença et al., 2010). In 2010, *O.* cf. *ovata* was also found in low densities in Penha and Bombinhas, in Santa Catarina (26°46′S; 48°39′W; Tibiriçá et al., 2010).

To date and along the Rio de Janeiro coastal zone, episodes of human illness have not been associated to the presence of *Ostreopsis* blooms. However, and as a possible consequence of marine aerosol exposure, beach users on the south coast of Bahia developed symptoms such as sore throat, headache, myalgy, fever sensation, cough and rhinorrea (Proença et al., 2010). Chemical analysis of extracts from *O.* cf. *ovata* from this location revealed a PLTX-like compound with a mass spectra characteristic of OVTX-a (Proença et al., 2010). However, no definite association between illness and blooms has been established for the events that were observed in the Bahia coastal area. Similar symptoms were described for exposed individuals along the Pernambuco coast, including human respiratory problems, heavy asthenia, high fever, muscular, articular and post orbital pain and occasional rashes on the thorax and the arms; and were thought to be linked with exposure due to blooms of the cyanobacterium *Trichodesmium erythraeum* (Satô et al., 1963).

The production of palytoxin and one of its analogue, 42-hydroxy-palytoxin by *Trichodesmium* was recently reported in New Caledonia by Kerbrat et al. (2011). The presence of the hydroxy palytoxin analogue in Brazilian coastal waters would require further investigations.

The toxicity of *O.* cf. *ovata* blooms and isolates from Brazil has been the focus of few recent analytical studies. An extracted compound closely resembling ostreocin D in two *O.* cf. *ovata* strains from Rio de Janeiro has been described by Riobó et al. (2006). Using LC–MS/MS, Tibiriçá et al. (2010) detected OVTX-a in an *O.* cf. *ovata* isolate from Santa Catarina, Brazil.

The aim of this study was to characterize the growth and to describe profiles and quantities of PLTX and OVTXs produced by two *O.* cf. *ovata* strains isolated from Rio de Janeiro, Brazil. In addition, we evaluated the morphometric features of field populations and isolates. Molecular analyses were performed to confirm species identification of one of the strains. The name *O.* cf. *ovata* was used in this study because of the lack of molecular data associated with strains from the type locality, following recommendations of Penna et al. (2010).

## 2. Materials and methods

### 2.1. Isolation of strains and establishing cultures

Two strains of *O.* cf. *ovata* (LCA-B7 and LCA-E7) were isolated from Armação dos Búzios (22°45′0″S, 41°53′0″W), Rio de Janeiro state (Fig. 1). The main physiographic characteristics of this tropical area are the rocky shores covered by granite boulders, ending in a sand bottom. The climate is semi-arid with a dry (May–August) and wet (December–February) period (CILSJ technical report, 2006). *O.* cf. *ovata* cells were collected from a sublittoral *Sargassum vulgare* bed growing approximately at 1 m depth in June 2006, and isolated using micropipette under an inverted microscope (ZEISS, Axiovert, Germany) and serial dilution. At the site of collection, seawater temperature varies between 21 and 28 °C and salinity between 34.1 and 37.1 (WTW thermo-salinometer).

Cultures were kept in sterile L-2/2 medium (Guillard, 1995) modified by omitting silicate, nickel, vanadium and chromium and prepared in GF/F filtered and autoclaved seawater. All stock cultures were maintained in a temperature-controlled cabinet at $24 \pm 2$ °C, with a light/dark cycle of 12 h:12 h and a photon flux density of 60 μmol $m^{-2}$ $s^{-1}$ (provided by cool-white fluorescent

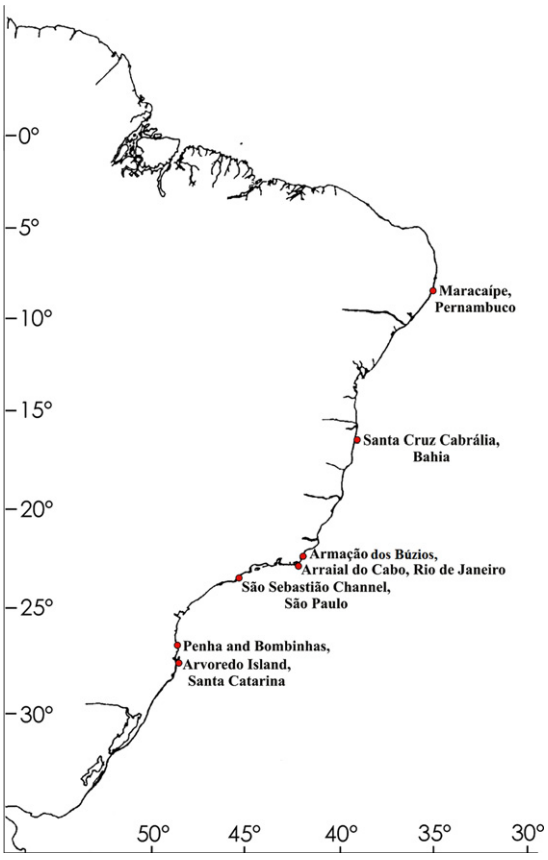


**Fig. 1.** Map showing locations where *O.* cf. *ovata* has been registered along the Brazilian coast.