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Comparative growth and toxin profile of cultured *Ostreopsis ovata* from the Tyrrhenian and Adriatic Seas

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ABSTRACT

Massive blooms of the benthic dinoflagellate *Ostreopsis ovata* Fukuyo have recently occurred along the whole Italian coastlines, both Tyrrhenian and Adriatic, resulting sometimes in benthonic biocenosis sufferings and, occasionally, in human health problems. In this work, two strains of *O. ovata* collected in 2006 along the Adriatic and Tyrrhenian coastlines and grown in culture were studied to characterize their growth and toxin profile. The two strains showed different cell volumes, the Adriatic strain being nearly twice bigger than the Tyrrhenian, but they had similar slow growth rates. Liquid chromatography-mass spectrometry (LC-MS) analyses indicated that both strains produce putative palytoxin (pPLTX) and ovatoxin-a (OVTX-a), a palytoxin-like compound presenting 2 oxygen atoms less than palytoxin.

Toxin content was determined at the end of the stationary and exponential growth phases and reached the highest value in the Adriatic strain at the end of the stationary phase, with concentrations of 353.3 μg l^{-1} for OVTX-a and 30.4 μg l^{-1} for pPLTX. Toxin released in the growth medium was also measured and resulted to be the highest at the end of the stationary phase, suggesting that a long lasting bloom could enhance the toxin content in the water and cause toxic effects in people inhaling the aerosol.

1. Introduction

Ostreopsis ovata is a benthic dinoflagellate, epiphytic on red and brown seaweeds and on rocks, sand, mussel shells and benthic invertebrates (Ballantine et al., 1988; Faust et al., 1996; Fukuyo, 1981; Totti et al., 2007). It generally blooms in shallow and sheltered waters, with low hydrodynamism (Vila et al., 2001), and covers emerging rocks and pebbly sea-bottom with a reddish-brown jelly film. *Ostreopsis* species are generally present in association with *Gambierdiscus toxicus, Coolia monotis* and *Prorocentrum* spp., so that in the past all these species were considered responsible for ciguatera fish poisoning in tropical and subtropical regions (Fukuyo, 1981). Since the beginning of the '70s, *Ostreopsis* spp. have been recognized as component of the Mediterranean benthic community. They were first observed in 1972 along the coasts of Villefranche-sur-Mer (Taylor, 1979) and, successively, in 1979, in Lebanese waters (Abboud-Abi Saab, 1989). The first report of *O. ovata* presence along the Italian coasts dates to 1994, when it was observed along the Tyrrhenian coastline (Lazio region, Tognetto et al., 1995). Since the end of the '90s, massive blooms of *O. ovata* were reported in a number of sites located along both the Tyrrhenian and the Adriatic coasts of Italy (Sansoni et al., 2003; Simoni et al., 2004; Gallitelli

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et al., 2005; Bianco et al., 2006; Zingone et al., 2006; Monti et al., 2007; Mangialajo et al., 2008; Totti et al., 2007). In most cases, *O. ovata* was present in association with other potentially toxic dinoflagellates such as *Prorocentrum lima* and *C. monotis* (Sansoni et al., 2003; Simoni et al., 2004). Intense *O. ovata* blooms sometimes resulted in extensive benthonic biocenosis sufferings and occasionally in human sufferings (Sansoni et al., 2003; Gallitelli et al., 2005). In summer 2005, the phenomenon broke out with alarming proportion along the Ligurian coasts (Ciminiello et al., 2006; Brescianini et al., 2006): an unusual proliferation of *O. ovata* (1.8×10^6 cells l^{-1}) occurred and caused an extensive environmental suffering involving mostly epibenthos, both sessile (cirripeds, bivalves, gastropods) and mobile (echinoderms, cephalopods, little fish). At the same time, hundreds of people required medical attention after exposure to marine aerosol during recreational or working activities on the beach and promenade of Genoa (Italy) (Ciminiello et al., 2006; Brescianini et al., 2006). The symptoms shown by all the patients included high fever associated to serious respiratory distress such as watery rhinorrhea, dry or mildly productive cough, bronchoconstriction with mild dyspnea and wheezes. Conjunctivitis and skin irritation was also observed in some cases and twenty people required extended hospitalization; most symptoms in humans disappeared as the population of *O. ovata* started fading away (Durando et al., 2007).

Following this event, the presence of *O. ovata* was carefully monitored along the whole Italian coasts and remarkable proliferations of *O. ovata* were reported in Liguria in 2006 (Ciminiello et al., 2008), in Sicily in 2005–2006 (Barone, 2007), along Campania coasts in 2007–2008 (ARPAC, 2008) and along Ancona coasts in 2006 (Totti et al., 2007) and 2007–2008 (Totti, personal communication). Bathing was forbidden in several Italian coastal areas and, thus, the number of people suffering from the toxic outbreaks was significantly limited in comparison to the 2005 event. These episodes caused great concern as *Ostreopsis* species, even from the Mediterranean Sea, are reported to produce palytoxin-like compounds (Ukena et al., 2001; Lenoir et al., 2004; Penna et al., 2005). Palytoxin (PLTX) is a complex polyhydroxylated water-soluble compound (Fig. 1) and one of the most potent non-protein marine toxins so far known (Moore and Bartolini, 1981; Uemura et al., 1981).

A method for determination of palytoxin, based on combination of liquid chromatography and mass spectrometry (LC-MS), was developed by Ciminiello and colleagues (Ciminiello et al., 2006, 2008) to investigate *O. ovata* toxin profile. Plankton samples collected along the Genoa coasts during 2005 and 2006 toxic outbreaks as well as *O. ovata* cultures were analysed by the newly developed method and the presence of two main toxins was highlighted in all the samples: putative palytoxin (pPLTX) and ovatoxin-a (OVTX-a), a palytoxin-like compound never reported so far. OVTX-a (MW = 2647.5; $C_{129}H_{223}N_3O_{52}$) presents 2 oxygen atoms less than PLTX (MW = 2679.5; $C_{129}H_{223}N_3O_{54}$) and its same A moiety (Fig. 1). In both natural plankton and cultured samples OVTX-a was by far the major toxin produced by the alga and, interestingly, the two compounds were produced by cultured cells approximately in the same ratio as in the natural sample, with pPLTX and OVTX-a contents of 0.55 and 3.85 pg $cell^{-1}$, respectively (Ciminiello et al., 2006, 2008).

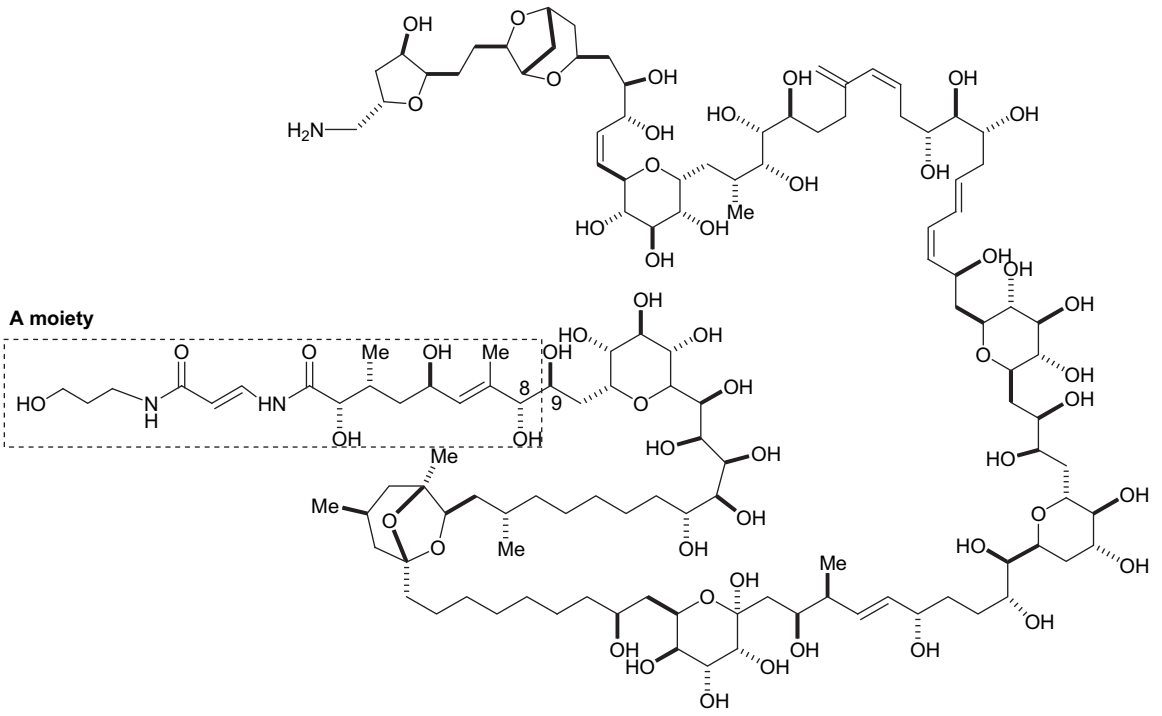

Fig. 1. Structure of palytoxin (PLTX) (MW = 2679.5; $C_{129}H_{223}N_3O_{54}$). Ovatoxin-a (OVTX-a) (MW = 2647.5; $C_{129}H_{223}N_3O_{52}$) presents 2 oxygen atoms less than PLTX and its same A moiety (Ciminiello et al., 2006, 2008).