ICES CM 2014/3712 H:13

Structural diversity of ovatoxins in *Ostreopsis* cf. *ovata* AZ strains and their impact on monitoring

Rachele Rossi (1), Adriana Zingone (2), Vittorio Soprano (1), and Takeshi Yasumoto (3)
(1) Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2, 80055, Portici, Italy
(2) Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy
(3) Japan Food Research Laboratories, 6-11-10 Nagayama, Tama-shi, Tokyo 206-0025, Japan.
Presenter contact details: rachele.rossi@izsmportici.it, phone 0039 081 7865 284

Summary
The structural diversity of ovatoxins (OvTXs) was studied in strains of *Ostreopsis* cf. *ovata* isolated from the Gulf of Naples (AZ strains) and grown in culture at Statione Zoologica A. Dohrn. ESILCTOF analysis, with complementary use of positive and negative ion modes revealed the occurrence of twelve palytoxin (PlTX) congeners. Five of them could be OvTX-a, OvTX-b, OvTX-c and OvTX-d/e, known in the literature but with undefined structures except for OvTX-a. Other congeners were indicated to be new having structural variations at the N-containing terminal units connected to the C1-carbon and/or at 115-NH2. The congeners seemed to share a similar carbon-chain backbone, a feature that should be taken into account when applying immunoassays for monitoring. The variances of the terminal units combined with the elusive LC behavior of some analogs should be taken into account when applying LC-MS/MS for determination. The occurrence of other unknown PlTX analogs was also detected, suggesting the need for further studies.

Introduction
The ovatoxins are palytoxin-like molecules produced by *Ostreopsis ovata*, a dinoflagellate that colonises macroalgae, seagrasses and benthic animals or grows directly on the substrate. In recent years, massive blooms of this species have become a threat along the Mediterranean coasts, where the toxins produced can affect human health. Species of the genus *Ostreopsis* are known to produce toxic substances including palytoxin, one of the most potent non-protein marine toxins, which was first isolated from the marine zoanthids *Palythoa* spp. (Moore and Bartolini 1981) followed by isolation of a congener from *Ostreopsis siamensis* (Uemura *et al.*, 1981), or palytoxin analogues such as ostreocin-D (Usami *et al.*, 1995; Ukena *et al.*, 2002). During the last years several ovatoxins have been found in *Ostreopsis* cf. *ovata* from the Mediterranean Sea (Ciminiello *et al.*, 2010; Rossi *et al.*, 2010; Ciminiello *et al.*, 2012) using LCMS for their identification. Recently, the complementary use of positive and negative ion mode in LCQTOFMS enabled to elucidate the structural characteristics of some ovatoxins (Uchida *et al.*, 2013). In this study, we identified 7 new ovatoxins in *O.* cf. *ovata* strains collected from the Gulf of Naples, and performed the structural assignment of their characterizing ions.

Materials and Methods
The strains of *Ostreopsis* cf. *ovata* were obtained from samples collected at the station Gaiola (AZ strain-D483), Ischia (AZ strain-NAP28) and Sorrento (AZ strain-NAP35) in the Gulf of Naples (Tyrrhenian Sea, Mediterranean Sea). They were collectively indicated as AZ strains to identify the source of the new structures. The LC consisted of an Agilent 1100 instruments equipped with a binary pump and an auto sampler. The mass spectrometer was an Agilent ESITOF G1969. Phenomenex Luna PFP(2) 3µ 150 x 2.00 mm was used for chromatographic separation.

Results and Discussion
The seven toxins identified in this study differed from all the ovatoxin-like compounds so far described. The complementary use of positive and negative ion mode enabled to infer the position of the distinctive changes, in the new molecules, which varied among them and from known palytoxins at the *N*-containing terminal unit connected to C1, *N*-containing terminal unit connected to C4', the hydroxyls on C42 and/or

C44, and C115-amine (Figure 1). Combination of the variances gave rise to diverse molecules. The production of the new molecules markedly varied among strains, which could be individually characterised based on their toxin profile. In fact some of the new molecules were only produced by one or two of the studied strains. The amount of these new toxins was presumed to be low as compared to the known ovatoxins but calculation of their relative abundance was impossible in the absence of the data for ionization rate. Nonetheless, their possible contribution to the overall toxicity of the strains should be taken into account. Numerous additional molecules were also found in the extracts, which warrant further studies for their identification and characterization. Our results clearly show the potentiality of the method used to detect the presence of novel biotoxins in microalgal species, thus contributing to a more effective prevention of human intoxications following the assumption of toxin-contaminated seafood. Further studies are under way to identify the numerous other unknown molecules in the extracts.

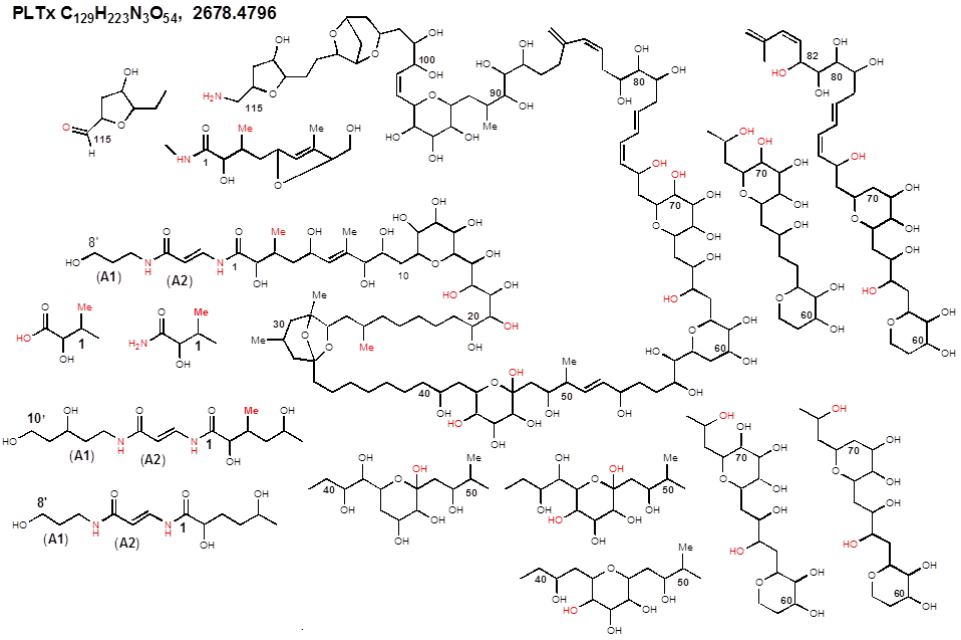

Figure. 1 Structural variations detected in the PLTX congeners

References

Ciminiello, P., Dell'Aversano, C., DelloIacovo, E., Fattorusso, E., Forino, M., Grauso, L., Tartaglione, L., Guerrini, F., Pistocchi, R. 2010. Complex palytoxin-like profile of *Ostreopsis ovata*. Identification of four new ovatoxins by high-resolution liquid chromatography/mass spectrometry. Rapid Commun. Mass Spectrom., 24: 2735-2744.

Ciminiello, P., Dell'Aversano, C., DelloIacovo, E., Fattorusso, E., Forino, M., Tartaglione, L., Battocchi, C., Crinelli, R., Carloni, E., Magnani, M., Penna, A. 2012. Unique toxin profile of a Mediterranean *Ostreopsis* cf. *ovata* strain: HRLC-MS(n) characterization of ovatoxin-f, a new palytoxin congener. Chem Res Toxicol 25(6):1243-1252.

Moore, R.E., Bartolini, G. Structure of palytoxin. 1981. J. Am. Chem. Soc., 103: 2491-2494.

Rossi, R., Castellano, V., Scalco, E., Serpe, L., Zingone, A., Soprano, V. 2010. New palytoxin-like molecules in Mediterranean *Ostreopsis* cf. *ovata* (Dinoflagellates) and in *Palythoa tuberculosa* by liquid chromatography-electrospray ionization time-of-flight mass spectrometry. Toxicon, 56: 1381-1387.

Uchida, H., Taira, Y., Yasumoto, T. 2013. Structural elucidation of palytoxin analogs produced by the dinoflagellate Ostreopsis ovata IK2 strain by complementary use of positive and negative ion liquid chromatography/quadrupole time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom., 27: 1999-2008.

Uemura, D., Ueda, K., Hirata, Y., Naoki, H., Iwashita, T. 1981. Further studies on palytoxin. II. structure of palytoxin. Tetrahedron Lett., 22: 2781-2784.

Ukena, T., Satake, M., Usami, M., Oshima, Y., Fujita, T., Naoki, H., Yasumoto, T. 2002. Structural confirmation of ostreocin-D by application of negative-ion fast-atom bombardment collision-induced dissociation tandem mass spectrometric methods. Rapid Commun. Mass Spectrom. 16: 2387-2393.

Usami, M.; Satake, M.; Ishida, S.; Inoue, A.; Kan, Y.; Yasumoto, T. 1995. Palytoxin Analogs from the Dinoflagellate *Ostreopsis siamensis*. J. Am. Chem. Soc. 117: 5389–5390.

Blooms of benthic dinoflagellates of the genus Ostreopsis (mainly O. cf. ovata and occasionally O. cf. siamensis) represent a serious concern for humans in.Â In this work, six strains of Ostreopsis sp. from Cyprus Island were analyzed through an integrated approach based on molecular, chemical, and eco-toxicological methods.Â Some variability in toxin profiles emerged: three strains produced ovatoxin-a (OVTX-a), OVTX-d, OVTX-e, and isobaric palytoxin, so far found only in O. cf. ovata; the other three strains produced only new palytoxin-like compounds, which we named ovatoxin-i, ovatoxin-j1, ovatoxin-j2, and ovatoxin-k. : Recurrent blooms of Ostreopsis cf. ovata have been reported in Brazil and the Mediterranean Sea with associated ecological, and in the latter case, health impacts. Molecular data based on the D1-D3 and D8-D10 regions of the LSU rDNA and ITS loci, and the morphology of O â€¦Â Ostreopsis cf. ovata (Dinophyceae) Molecular Phylogeny, Morphology, and Detection of Ovatoxins in Strains and Field Samples from Brazil. Toxins (Basel). 2020 Jan 22;12(2):70. doi: 10.3390/toxins12020070.Â Toxin profiles and quantities of PLTX and their analogues; OVTXs; contained in cells from two clonal cultures and two field blooms from Rio de Janeiro were investigated.Â Morphometric analysis of different strains and field populations from diverse locations were compared. O. cf. ovata can produce palytoxin, ovatoxins, and mascarenotoxins [30â€“34] and is responsible for recurrent toxic blooms in the Mediterranean Sea (up to 1.8 x 106 cells.L-1) with notable effects on socio-eco-nomic activities and public health [35,36]. P. lima, a cosmopolitan toxic dinoflagellate, is also known to produce several toxins, such as okadaic acid, dinophysistoxins, prorocentrolide, and prorocentin [37â€“39], that can cause diarrhetic shellfish poisoning episodes. Previous studies have demonstrated that O. cf. ovata produces analogues of palytoxin (ovatoxins and a putative palytoxin), one of the most potent marine toxins.Â A small scale monitoring study of marine aerosol carried out along the Tuscan coasts (Italy) in 2009 and 2010 is reported. Aerosols were collected concurrently O. cf. ovata blooms and they were analyzed by both PCR assays and LC-HRMS technique. The results, besides confirming the presence of O. cf. ovata cells, demonstrated for the first time the occurrence of ovatoxins in the aerosol at levels of 2.4 pg of ovatoxins per air liter.Â Ovatoxin-a, the major component of most O. cf. ovata strains, has been recently isolated from algal cultures and. structurally elucidated based on both NMR. 12,13. Variability in Toxin Profiles of the Mediterranean Ostreopsis cf. ovata and in Structural Features of the Produced Ovatoxins. Authors: Luciana Tartaglione Emma Dello Iacovo Antonia Mazzeo Silvia Casabianca Patrizia Ciminiello Antonella Penna Carmela Dell'Aversano. Environ Sci Technol 2017 Dec 22;51(23):13920-13928.Â Fifty-five strains of Ostreopsis were collected in the Mediterranean Sea and analyzed to characterize their toxin profiles. All the strains were grown in culture under the same experimental conditions and identified by molecular PCR assay based on the ITS-5.8S rDNA. A liquid chromatography-high resolution multiple stage mass spectrometry (LC-HRMS) approach was used to analyze toxin profiles and to structurally characterize the detected toxins.