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Detection of palytoxin-like compounds by a flow cytometry-based immunoassay supported by functional and analytical methods

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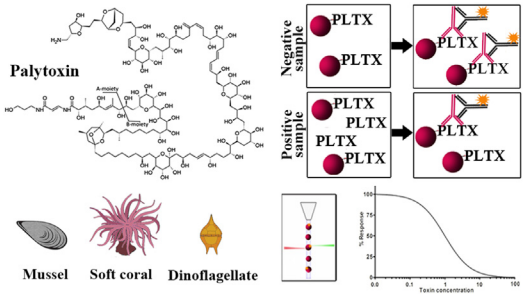
HIGHLIGHTS

- Microsphere-based flow cytometry method for the detection of PLTX-like molecules.
- Sensitive, easy-to-perform, semi-quantitative screening technique.
- Useful for the detection of PLTX-like molecules in coral and dinoflagellate samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Palytoxin (PLTX) is a complex marine toxin produced by zoanthids (i.e. *Palythoa*), dinoflagellates (*Ostreopsis*) and cyanobacteria (*Trichodesmium*). PLTX outbreaks are usually associated with Indo-Pacific waters, however their recent repeated occurrence in Mediterranean–European Atlantic coasts demonstrate their current worldwide distribution. Human sickness and fatalities have been associated with toxic algal blooms and ingestion of seafood contaminated with PLTX-like molecules. These toxins represent a serious threat to human health. There is an immediate need to develop easy-to-use, rapid detection methods due to the lack of validated protocols for their detection and quantification. We have developed an immuno-detection method for PLTX-like molecules based on the use of microspheres coupled to flow-cytometry detection (Luminex 200™). The assay consisted of the competition between free PLTX-like compounds in solution and PLTX immobilized on the surface of microspheres for binding to a specific monoclonal anti-PLTX antibody. This method displays an IC_{50} of 1.83 ± 0.21 nM and a dynamic range of 0.47–6.54 nM for PLTX. An easy-to-perform extraction protocol, based on a mixture of methanol and acetate buffer, was applied to spiked mussel samples providing a recovery rate of 104 ± 8% and a range of detection from 374 ± 81 to 4430 ± 150 μg kg^{-1} when assayed with this method. Extracts of *Ostreopsis* cf. *siamensis* and *Palythoa tuberculosa* were tested and yielded positive results for PLTX-like molecules. However, the data obtained for the coral sample suggested that this antibody did not detect 42-OH-PLTX efficiently. The same samples were further analyzed using a neuroblastoma cytotoxicity assay and UPLC-IT-TOF spectrometry, which also pointed to the presence of PLTX-like

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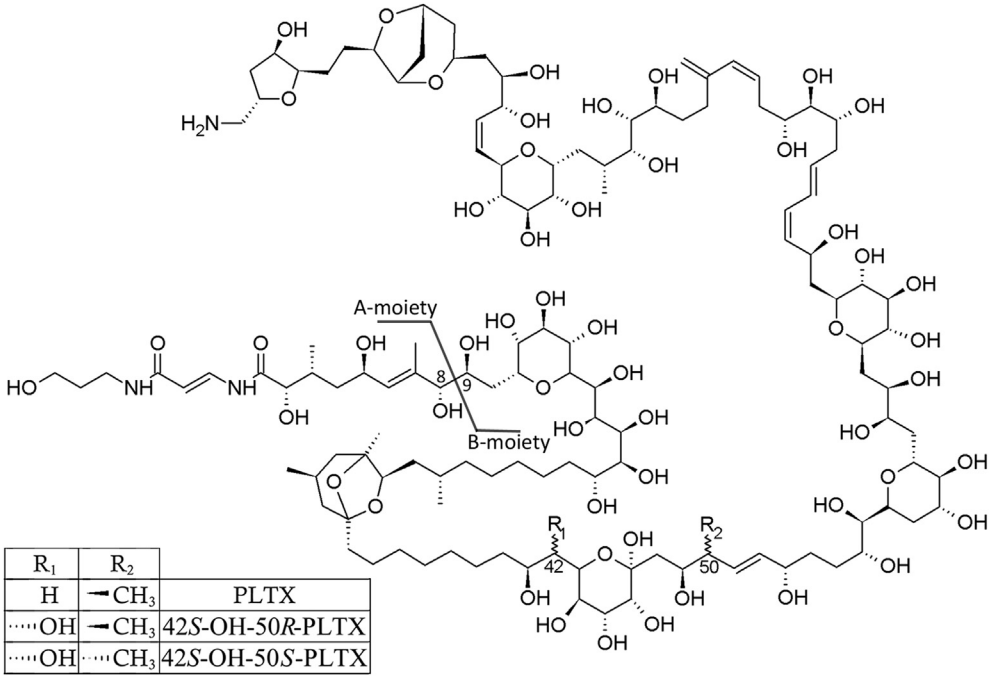
compounds. Therefore, this single detection method for PLTX provides a semi-quantitative tool useful for the screening of PLTX-like molecules in different matrixes.

1. Introduction

Palytoxin (PLTX) is a marine toxin considered as one of the most potent and complex natural non-protein compounds (Fig. 1). In the 1960's polyps of *Palythoa* collected in Hana (Maui) were used for the first isolation of PLTX [1]. Its chemical characterization, carried out in parallel by two research groups, took more than a decade [2,3]. Since then, many PLTX-like molecules have been described and associated with several producer organisms: zoanthids (*Palythoa* and *Zoanthus*), dinoflagellates (*Ostreopsis*) and cyanobacteria (*Trichodesmium*) [4–7]. PLTX and 42-hydroxy-palytoxin (42-OH-PLTX) were identified from different species of zoanthids, together with other minor toxins [7–9]. PLTX and ovatoxins (from -a to -h, and some isomers) were detected in different strains of *Ostreopsis ovata* [10–14]. In *Ostreopsis siamensis* ostreocin-d was described as the major toxin [15], while mascarenotoxins (-a and -b) were extracted from *Ostreopsis mascarenensis* [6].

PLTX-like molecules were usually associated with Indo-Pacific waters. However, recent, repeated appearances in Mediterranean–Atlantic coasts of Europe point to their global distribution [16–18]. Additionally, these toxins have been detected in different species of fish, crustaceans, mollusks and echinoderms suggesting their entrance into the trophic food web [16]. Actually, human sickness and fatalities have been associated with the uptake of seafood contaminated with PLTX, and skin and respiratory affections have been related to contact with PLTX-producer organisms, usually during dinoflagellate blooms or manipulation of aquarium zoanthids [19]. Currently, there are no regulated legal limits or validated detection methods for this class of toxins. However, the presence of PLTX-like compounds in seafood and marine aerosols [16,20] indicates that these toxins are a potential sanitary risk, and generates the need for establishing limitations on their amount in marine products destined for human consumption and monitoring their presence in recreational waters. The lack of certified standard materials has hampered the validation of detection methods; nevertheless, PLTX-like compounds have been commonly detected by mouse bioassay (MBA), and different chemical and biomolecular methods [21]. Although the initial symptoms observed in MBA when injecting PLTX differ from others caused by common marine toxins [22], this method presents ethical issues and technical disadvantages. Liquid chromatography coupled to ultraviolet, fluorescent or mass spectrometry detection have been widely used for the study and identification of PLTXs [21]. Fast-atom bombardment and nuclear magnetic resonance, among others, have been crucial for the elucidation of the chemical structure of PLTX and a few analogues [21]. However, the cost of these analytical methods, the complexity of these compounds and the need of highly trained personnel have precluded their use as extended routine detection tools for PLTX-like toxins, and have prompted the development of alternative methods as screening assays. Several cell types (i.e. neuroblastoma cells or erythrocytes) have been used for the development of cytotoxicity assays [23–25] based on the capability of PLTX to transform the Na^+/K^+-ATPase into a non-specific ion channel, triggering a disturbance of the cellular ion homeostasis [26]. Also a spectroscopic technique has been developed based on the measurement of changes in fluorescence polarization by PLTX binding to fluorescently labeled Na^+/K^+-ATPase [27]. The generation of



R_1	R_2	
H	CH_3	PLTX
OH	CH_3	42*S*-OH-50*R*-PLTX
OH	CH_3	42*S*-OH-50*S*-PLTX

Fig. 1. Chemical structures of PLTX and 42-OH-PLTX from *Palythoa tuberculosa*.