Development and characterization of a cell-based ELISA for palytoxin quantitation

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Palytoxin (PLTX) and its analogs, initially identified in corals of the genus *Palythoa*, and later in *Ostreopsis* dinoflagellates and *Trichodesmium* cyanobacteria, is considered one of the most toxic non-proteinaceous marine compounds. The main problem associated to these toxins is their accumulation in marine edible organisms and their possible entrance in the human food chain. Indeed, a series of human poisonings, sometimes with fatal outcomes, were ascribed to these compounds in tropical and subtropical areas. In the Mediterranean Sea, PLTXs have been recently detected also in edible mollusks and echinoderms, concomitantly to *Ostreopsis* blooms, even though no foodborne poisonings have been associated to PLTXs in this area, so far. Although PLTXs are not currently regulated in seafood, the European Food Safety Authority (EFSA) suggested a maximum limit of 30 μg PLTXs/Kg of shellfish meat, recommending also the development of rapid, specific and sensitive methods for PLTXs detection in contaminated seafood. Thus, the high affinity binding of PLTX to HaCaT keratinocytes was exploited to develop and characterize a cell-based ELISA for the toxin quantitation in mussels.

Evaluating different parameters (incubation temperature of primary and secondary antibodies, fixing and blocking agents, use of different cell lines), the developed assay for a sensitive PLTX detection was carried out exposing HaCaT cells to PLTX (working range from $1.4x10^{-11}$ to $1.1x10^{-9}$ M) and fixing them for 30 min with 4% paraformaldehyde. Cells were then blocked for 30 min in TBB buffer (Trizma Base 50 mM, NaCl 0.15 M, BSA 2%, 0.2% Tween 20, pH 7.5) containing 10% horse serum and the toxin detected after exposure to monoclonal anti-PLTX mouse antibody (0.5 μg/mL, for 1 h at 50 °C) followed by HRP-conjugated secondary anti-mouse IgG antibody (1:6000, for 1 h at 50 °C). The colorimetric reaction was then started with 60 μL 3,3′,5,5′-tetramethylbenzidine substrate, stopped after 60 min by 1 M 30 μL H_2SO_4. The absorbance was then read at 450 nm. Under these conditions, the limit of PLTX detection (LOD) and quantitation (LOQ) were $1.2x10^{-11}$ M (equal to 32.2 pg/mL) and $2.8x10^{-11}$ M (equal to 75.0 pg/mL), respectively, with good accuracy (bias=2.5%) and repeatability (RSDr equal to 15% and 9% for interday and intraday repeatability, respectively). With the aim to evaluate the applicability of the assay to quantify PLTX in seafood (i.e. *Mytilus galloprovincialis*), the influence of 80% aqueous methanol mussel extract on PLTX quantitation was studied. The minimum extract dilution that did not interfere with the assay was 1:10 and a provisional LOQ for PLTX in mussel was equal to $3.4x10^{-11}$ M. Considering the extraction procedure and the 1:10 dilution, this value correspond to 9.1 μg PLTXs/kg shellfish meat, a level below the maximum limit suggested by EFSA (30 μg PLTXs/Kg of shellfish meat).