132
PALYTOXIN DETECTION BY ELECTROCHEMICAL SENSOR: IMPROVEMENT OF EXTRACTION PROCEDURES AND EVALUATION ON DIFFERENT SHELLFISH SPECIES

Cozzi L.[1], Volpe G.[2], Petropoulos K.[2], Suffredini E.[1], Palleschi G.[2]

[1]Istituto Superiore di Sanità, Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare ~ Roma ~ Italy, [2]Università di Roma Tor Vergata, Dipartimento di Scienze e Tecnologie Chimiche ~ Roma ~ Italy

Keywords: Palytoxin, shellfish, sensor, hemolysis

INTRODUCTION:

Over the past few decades, the occurrence of harmful algal blooms has increased both in frequency and in geographic distribution in many areas of the world. This resulted is negative effects both on public health and on economy. Several algal species, in fact, can produce potent toxins which affect human health through the consumption of contaminated seafood or aerosol exposure. Palytoxin (PlTX) is one of the most potent marine toxins known and is produced, together with its analogues (PlTXs), by benthic dinoflagellates belonging to the genus Ostreopsis, a genus recently found also in the Mediterranean Sea. Occurrence of Ostreopsis spp. may therefore result in PlTX presence in seafood (250 µg/kg proposed regulatory limit) and, in order to prevent sanitary risks, rapid and sensitive monitoring methods for PlTX-group toxins are needed.

MATERIALS AND METHODS:

We recently developed an electrochemical biosensor method for PlTX detection (1). The method is based on a mediated amperometric measure of the lactic dehydrogenase (LDH) released by the PlTX-induced hemolysis of sheep erythrocytes. In method development different extraction procedures from mussels were compared (1, 2, 3) and some of them showed a strong matrix effect on the assay, with an evident inhibition of the PlTX-induced hemolysis even in presence of high concentrations of toxin. In this work we assessed the performance of a different, simple extraction procedure for shellfish testing, evaluating specifically its efficiency in reducing the matrix effect on the bioassay. The procedure included the following steps: blending of 10 g of whole shellfish tissue with 90 ml of PBS, centrifugation (4800 ×g at 4°C for 20 min), filtration through a 0.22 µm filter, shaking with chloroform (1:1 v/v) for 15 min followed by centrifugation (12500 ×g for 5 min) and collection of the aqueous phase and, finally, dilution 1:50 of the retained suspension in PBS.

Analysis was performed on two different shellfish species (mussels and clams) to assess the response of the extraction to variations of tissue composition. Samples were prepared by spiking the shellfish extracts with different concentrations of PlTX (0.2, 0.4, 0.8, 1.6, 3.12 ng/ml) and were compared to same toxin concentrations in PBS.

RESULTS:

The results of the experiments showed that the proposed extraction procedure allowed an almost complete removal of the matrix effect. The amperometric signal obtained by the haemolytic-enzymatic assay on shellfish extracts showed a significant agreement with the results of the blank sample (Fig. 1). The concordance of the results on mussels and clams confirmed the performance of such procedure on different kinds of shellfish species.

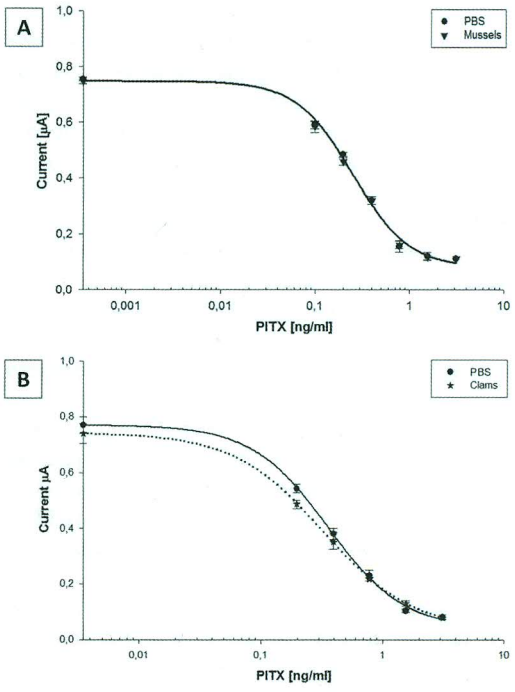

Fig. 1: Evaluation of matrix effect: extracts from mussels (panel A) and clams (panel B) compared to PBS

DISCUSSION AND CONCLUSIONS:

The proposed extraction procedure, by reducing the matrix effect, allows a significant improvement of the samples preparation for PlTX detection by electrochemical sensor. Additionally, the procedure is simple to perform, rapid and cost-effective, therefore showing fitness for the use in a rapid detection method. Further experiments are ongoing to quantitatively assess the recovery of PlTX from matrix.

REFERENCES:

1) G. Volpe, L. Cozzi, D. Migliorelli, L. Croci, G. Palleschi (2014). Development of a haemolytic–enzymatic assay with mediated amperometric detection for palytoxin analysis: application to mussels. Analytical and Bioanalytical Chemistry, 406 (9-10): 2399-2410.

2) Ledreux A, Krys S, Bernard C (2009). Suitability of the neuro-2a cell line for the detection of palytoxin and analogue (neurotoxic phycotoxin). Toxicon 53:300–308

3) Milandri A, Ceredi A, Riccardi E, Gasperetti L, Susini F, Casotti M, Faiman L, Pigozzi S (2012). Proceedings of the 14th International Conference on Harmful Algae. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO