ALARMTOX: Assays and biosensors for okadaic acid and microcystin based on genetically-engineered protein phosphatases

Helena Eixarch[1]; Diana Garibo[1]; Pablo de la Iglesia[1]; Esther Barber[1]; Jorge Diogène[1]; Margarita Fernández-Tejedor[1]; Cintia Flores[2]; Audrey Sassolas[4]; Gaelle Catanante[4]; Alain Guibert[3]; Valérie Dumont[3]; Josep Caixach[2]; Jean-Louis Marty[4]; Mònica Campàs[1]

[1]IRTA, Ctra. Poble Nou, km. 5.5, 43540 Sant Carles de la Ràpita, Spain; [2]IDAEA, CSIC, C. Jordi Girona, 18-26, 08034 Barcelona, Spain; [3]CRITT-BioIndustries, INSA,135, Av. de Rangueil, 31077 Toulouse Cedex, France; [4]BIOMEM-IMAGES, UPVD, 52 Av. Paul Alduy, 66860 Perpignan Cedex, France

monica.campas@irta.cat

Alarmtox

www.alarmtox.net

INTRODUCTION

Several microalgae species are able to produce toxins whose presence has negative social-economical effects. Food and health safety is compromised through contamination of shellfish, drinkable water and nutritional algae, or by direct contact with them; their correct evaluation, which may eventually result in the closure of coastal shellfish production areas or aquatic recreational areas, favors consumer protection and the long-term sustainability of aquaculture.

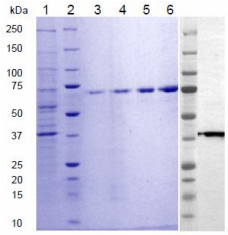
THE PROJECT

The main objective of the ALARMTOX project is to develop and validate assays and biosensors for biotoxin detection that guarantee the quality of continental waters and aquaculture products. These new technologies should be more specific, more sensitive, faster and less expensive than the currently used biological and analytical methods. A multi-disciplinary approach will be followed, exploiting the expertise of each one of the partners.

STEP 1: Production of protein phosphatases

Protein phosphatases (PPs) have been genetically engineered to improve their stability and their sensitivity to microcystins (MCs), okadaic acid (OA) and derivatives. The PPs produced at CRITT-Bio-Industries-INSA have been purified (figure 1) and will be used in the subsequent steps of the project. There PP has an His tail that will allow their conjugation to magnetic particles via coordination chemistry.

Figure 1: (Left) Coomassie blue stained SDS-PAGE gel. Lane 1: Purified genetically engineered PP; Lane2: Molecular weight marker; Lanes 3 to 6: BSA. (Right) Western blot of purified PP, as detected by an anti-His antibody.



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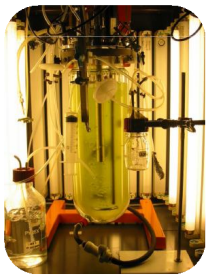


STEP 2: Development of colorimetric assays and electrochemical biosensors

The extent of PP activity inhibition by MCs and OA is proportional to the amount of toxin present in the sample (figures 2 and 3). This is the basis for both the colorimetric assay and the electrochemical biosensor. At present, the colorimetric approach provides lower limits of detection than the electrochemical one, both with the enzyme in solution or immobilised into polymers. It is expected to improve the sensitivity by immobilising the enzyme onto magnetic particles.

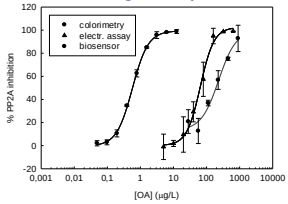
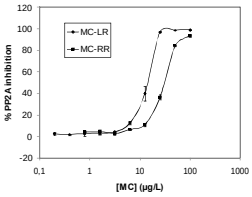


Figure 2 (left): OA calibration curve obtained with i) the colorimetric assay (PP in solution), ii) the electrochemical assay (PP in solution), and iii) the biosensor (PP immobilised by entrapment). p-Nitrophenyl phosphate is hydrolysed by PP and its coloured product is detected at 405 nm or α-naphthyl phosphate is hydrolysed by PP and its electroactive product is detected by Differential Pulse Voltammetry.

Figure 3 (right): MC calibration curve obtained with the colorimetric assay (PP in solution). p-Nitrophenyl phosphate is hydrolysed by PP and its coloured product is detected at 405 nm.



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STEP 3: Validation of assays and biosensors

Several public institutions and private enterprises are providing water, microalgae and shellfish samples from a great variety of ecosystems (figure 4), which are being tested with the developed assays and biosensors. Their performance and validity is being checked by comparison with LC-MS/MS analysis. Matrix effects have also been evaluated (figure 5).

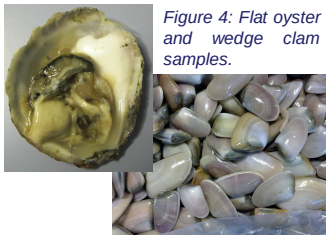
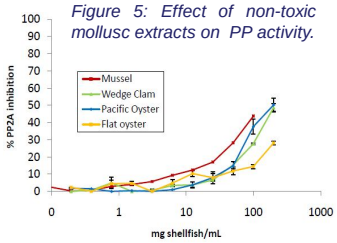


Figure 4: Flat oyster and wedge clam samples.

Figure 5: Effect of non-toxic mollusc extracts on PP activity.



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