Development of biosensors for the assessment of water and shellfish safety: the ALARMTOX project

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INTRODUCTION

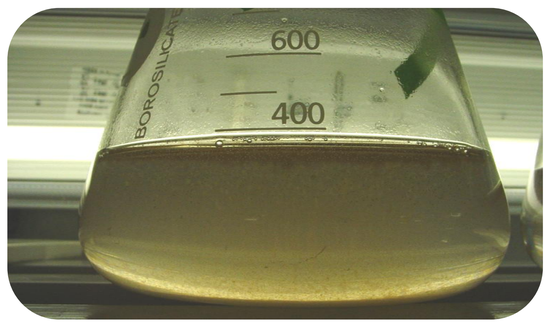
Toxin-producing microalgae species have a negative influence in several areas, such as food safety, health, environment and socio-economy. Contamination of shellfish, drinkable water and nutritional algae or direct exposure to toxins may cause general illnesses and diseases both in humans and animals. Moreover, presence of toxins in shellfish harvesting areas is a threat for public safety, and their correct evaluation, which may eventually result in the closure of production areas, favours consumer protection and the long-term sustainability of aquaculture.

THE PROJECT

The ALARMTOX project is focused on the development and validation of assays and biosensors for the detection of aquatic biotoxins, with the aim of establishing new technologies to guarantee the quality of continental waters and aquaculture products. These technologies should be more specific, more sensitive, faster and less expensive than the currently used biological and analytical methods.

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 produced PPs have been purified and will be used in the subsequent steps of the project.



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. 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 with the enzyme in solution or immobilised into polymers. It is expected to improve the sensitivity by immobilising the enzyme through magnetic particles.

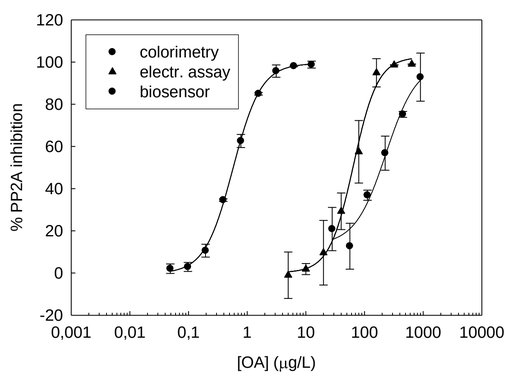
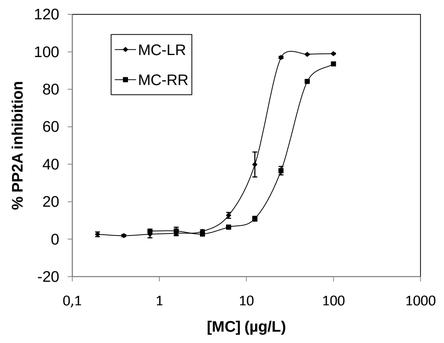


Figure 1 (left): OA calibration curve obtained with i) the colorimetric assay (PP in solution), ii) the electrochemical assay (PP in solution), and iii) the biosensor (PP immobilised by entrapment). p-Nitrophenyl phosphate is hydrolysed by PP and its coloured product is detected at 405 nm or α-naphthyl phosphate is hydrolysed by PP and its electroactive product is detected by Differential Pulse Voltammetry.

Figure 2 (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.



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, which are being tested with the developed assays and biosensors. Their performance and validity will be checked by comparison with LC-MS/MS analysis. Matrix effects have also been evaluated (figure 4).

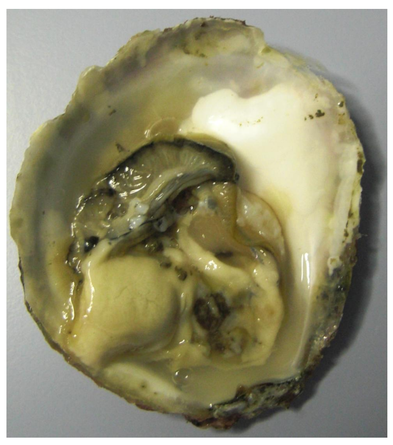


Figure 3 (left): Flat oyster sample.

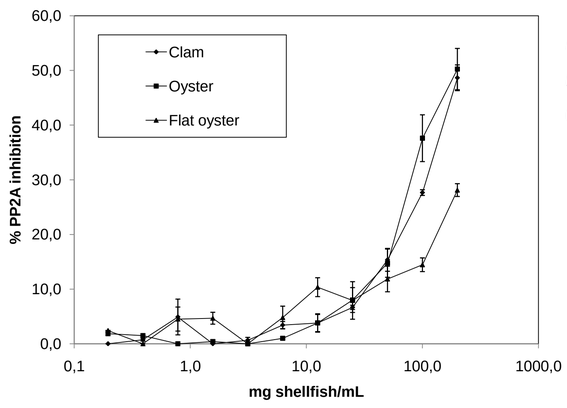


Figure 4 (right): Effect of non-toxic mollusc extracts on the PP activity.

ALARMTOX is a project co-financed by the European Union through the Interreg IV B SUDOE (South-West Europe) Territorial Cooperation Programme. It is integrated into the first priority of the Programme: “Promotion of innovation and constitution of stable cooperation networks in technological matters”. The presented opinions only compromise the partners and, consequently, in no way represent the opinion of the Territorial Cooperation Programme’s management bodies.