



Work Package 3: Biosensors

Introduction

The main objective of the BioCop project is to develop the existing methodologies, aimed to improve chemical contaminant monitoring in food. New technologies, including optical and electrochemical biosensors, are being developed by Work Package 3 to fulfil this objective.

Work Package Progress and Results

Optical biosensors

In the area of food safety, optical biosensors based on surface plasmon resonance (SPR), are used to rapidly quantify chemical contaminants in food. The biosensor methodology is based on quantifying contaminants by capturing them by specific receptors on a sensing area. Currently these biosensors are used to monitor veterinary drug residues and pathogens including *Trichinella* and *Salmonella* using Biacore Q® or Q100 together with Qflex® kits.

Shellfish poisons and antibiotics

Within the project, two new assay kits for the detection of paralytic shellfish poisons (saxitoxins) and the entire family of fluoroquinolone antibiotics were developed in collaboration with Work Packages 5 (Phycotoxins) and 8 (Therapeutics).

Hormone growth promoter biomarkers

The above mentioned biosensor technology is also used as a prototype instrument, designed to quantify 16 biomarkers simultaneously. The selected biomarkers are proteins that have a distorted concentration in cattle plasma due to exposure to growth hormones. The biomarker selection and assay development process is performed by Work Package 2. Results from these biomarker assays will define the probability of an animal having been illegally treated

with growth hormones. WP8 has an important role in the evaluation of the biomarker-based approach in monitoring the biological effect of growth promoters.

SPR/MS Coupling

Coupling SPR biosensors with mass spectrometry (MS) systems is a new technique used to identify known and unknown bioactive substances. These substances are recognized as non-compliant in newly developed optical biosensor screens. This methodology is successfully applied to detect fluoroquinolone antibiotics and paralytic shellfish toxins.

The compounds identified in BioCop are low molecular weight drugs, toxins and contaminants. Because of low concentrations of residues in food and excreta, MS-based techniques are generally preferred over alternatives such as nuclear magnetic resonance (NMR) spectroscopy. SPR-based biosensors are coupled with mass spectrometry systems in a serial and parallel manner, as described below.

Serial: The suspect sample is applied to a liquid chromatography column and the effluent is split between two identical 96-well plate fraction collectors. One of the plates is then subjected to the SPR assay for bioactivity measurement and generates a "biogram" of the column effluent.

This biogram will direct the MS identification efforts to the relevant sample fractions in the duplicate plate.

Parallel: A more advanced and challenging SPR/MS coupling is achieved by a direct (parallel) coupling. With this method, the suspect sample is first screened with a bio-assay and non-compliant samples are further processed by capturing the compounds on a sensor surface (all using Biacore® 3000). After the on-chip purification step, samples are eluted from the chip directly into a liquid chromatography-MS system for compound identification.





Electrochemical immunosensors for trichothecenes detection

In the framework of WP3, the University of Rome, Tor Vergata (URTV) in collaboration with Work Packages 10 (Mycotoxins) and 4 (Binders) have developed electrochemical immunosensors for the detection of HT-2, T-2 and DON trichothecenes.

The electrochemical immunosensors are based on indirect competitive formats, the use of magnetic beads, screen printed electrodes and a portable instrument (PalmSens) thus allowing the in-situ analysis of toxins. The immunosensor used for the detection of deoxynivalenol (DON) is based on the use of a Fab fragment obtained by the University of Turku. The Immunosensor measures DON in breakfast cereal products and baby food samples at regulatory levels.

In the case of type-A trichothecenes, the monoclonal antibody for T-2 and HT-2 toxins was produced by WPI0. Extensive in-house tests were carried out and demonstrated the potential applicability of the present method as a screening tool for T-2 and HT-2 detection.

The electrochemical immunosensor is a valid potential alternative to optical based methods. As a result, the developed immunosensors will be tested in an inter-laboratory pre-validation test. From this perspective, three practical demonstrations have been carried out by URTV and WPI0 partners during the last 6 months. The Standard Operation Protocol (SOP) of the method was drafted in CEN format.

Benefits of the BioCop Project

Consumer

The new sensor technologies provide accurate and rapid quantification of a number of food contaminants. For consumers this means that food products can be screened more thoroughly before they reach the customer. SPR/MS coupling provides technology for rapidly identifying novel contaminants.

Food Industry

Rapid and accurate analysis provide the means to release food products faster to the market.

Scientist

New technologies such as biomarker-based screening of growth hormones and SPR/MS coupling provide information to scientists to study the biological effect of growth hormones and identify novel contaminants, respectively.

Training/Workshops

Demonstration activities have been carried out during the World Mycotoxin Forum (2008), the Biocop Open Day (2008) and the NRL meeting (2009) to both train and disseminate Electrochemical immunosensors. A Protocol Video describing the protocol measurement is also available.

Future Activity

Future activities are primarily focused on supporting validation of the new technologies and methodologies as carried out in other work packages (Shellfish poisons assay in WP5, growth hormone abuse and fluoroquinolone assay in WP8, and electrochemical trichothecenes immunosensor assay in WPI0)





Frequently Asked Questions

Question:

- Q What is the advantage of monitoring protein expression levels rather than growth hormone residue levels ?

Answer:

- A The main difference, and advantage, is that the proteomics-based concept focuses on a measuring effect rather than measuring single target compound concentrations. This effect-oriented approach has a clear advantage for the monitoring of traces of specific hormones, in terms of throughput and the possibility to detect newly developed designer steroids and hormone cocktails over the currently residue monitoring approach.

Question:

- Q Why is parallel coupling more challenging than serial coupling?

Answer:

- A In parallel coupling, the SPR-based biosensor is not only used for screening suspect samples but also for affinity based on-chip purification and concentration. After this purification and concentration step, samples are on-line injected into the LC-MS system for identification. This on-line processing can be performed with relatively small sample quantities but requires compatibility of solvents used in the on-chip purification and LC-MS analysis steps.



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