Work Package 10: Mycotoxins

Introduction

Workpackage 10 focuses on mycotoxins (toxic metabolites produced by fungi). Mycotoxins have been included in the BioCop project from the start, because of their wide occurrence in agricultural commodities and the significance of their hazards to human and animal health. In Europe, toxins produced by Fusarium species are of particular significance in agriculture. A major class of Fusarium toxins are the trichothecenes, which can be found in cereals.

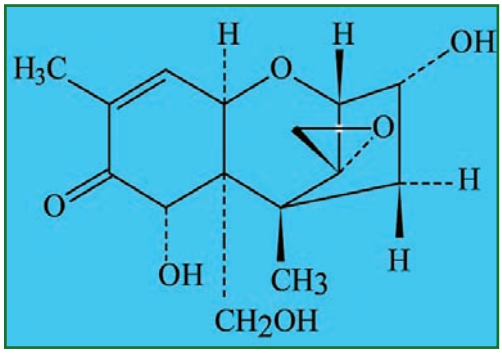

Figure 1: Chemical structure of trichothecenes

Over 150 trichothecenes are known, of which deoxynivalenol (DON) is the most well-known. DON leads to undesirable effects on the immune system, to growth retardation in children and to feed refusal by pigs. Other trichothecenes of major significance are T-2 and HT-2 toxins. The potential risks of these compounds have been assessed by several international committees, including the EC's Scientific Committee on Food and the WHO/FAO Joint Expert Committee on Food Additives. Detailed harmonised regulations and limits for deoxynivalenol in cereals, cereal-based products and baby food have recently come into force in the EU and they are expected to be soon introduced for T-2 and HT-2 toxins

The expansion in the European mycotoxin regulatory area demands continuous activities to develop and validate suitable high-throughput and low-cost methodologies, to make enforcement of the regulations possible. In comparison, current reference methods for trichothecenes (based on chromatographic procedures) are relatively time-consuming and costly. In Biocop, trichothecene detection methods have been developed based on sensors and transcriptomics technologies.

Work Package Progress and Results

Pure toxins, suitable antibodies, well-characterised naturally-contaminated test materials, efficient extraction and effective purification techniques have been produced and developed respectively.

Figure 2: Breakfast cereal test materials, characterised for trichothecenes

The electrochemical immunosensor technique for the determination of DON and for T-2/HT-2 toxins has evolved from concept to reality as a result of combined efforts of work packages 3 and 10. Methods for DON and for T-2/HT-2 toxins have been extensively in-house tested for various cereal products and yielded good results, i.e. limits of detection at target levels, and precise and accurate performance characteristics Details have become available to the consortium in a variety of BioCop reports, and a draft Method Protocol has been produced in CEN format.

In-house validation and testing of surface plasmon resonance sensor methodology has been completed for T-2/HT-2 toxins in wheat, breakfast cereals and baby food. The results showed low limits of detection and recovery values around 100 %. Validation and in-house testing of the SPR methodology is ongoing for DON.

In interaction with work package 1, efforts to determine T-2/HT-2 in cereals with transcriptomics have shown the potential of this new technique to detect these toxins at low levels of occurrence. The quantitative aspects of the technique are under investigation, where special attention is focused to sensitivity. Personnel exchange programs between BioCop chemical and cell-focused laboratories has helped the project to enable the method to be performed in different laboratories

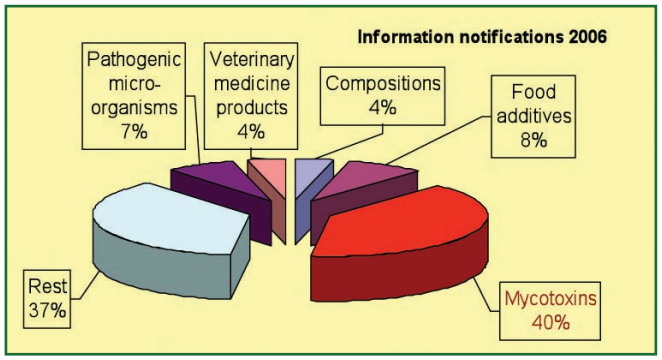

Figure 3: RASFF Information notifications in 2006

Benefits of the BioCop Project

Consumer

Currently mycotoxins form the largest group of information notifications on risks to human health,in the EU Rapid Alert System for Food and Feed (RASFF).

These notifications refer to imports, sampled and analysed at the outer borders of the EU. This is remarkable as the relevant regulations require each shipment of these materials to be accompanied by a certificate of analysis detailing the mycotoxin content of the shipment. The advent of new high-throughput, low-cost and validated BioCop methods to determine trichothecenes may contribute to more reliable analyses carried out both by the exporting and importing countries, and therefore lowering the risk of consumers being exposed to unacceptable levels of these toxins.

Food Industry

Increasingly, rapid test methods, including those for mycotoxins, are being used by food businesses as a tool to assist them in ensuring that "food placed on the market shall be safe". Within the European Union, ensuring that food is safe in terms of mycotoxin contamination means - in the case of a number of mycotoxin/food matrix combinations - ensuring that mycotoxin residues do not exceed regulatory limits. Frequent testing, as part of the verification process in food safety management systems, with rapid and reliable methodology is of particular importantance where the incidences and levels of contamination are variable. Such a state of affairs can arise in the case of mycotoxins produced by Fusarium spp. mycotoxins, in particular trichothecenes as deoxynivalenol and T-2 and HT-2 toxins. BioCop methods are specifically directed to determine these toxins.

Scientist

BioCop mycotoxin methods are based on novel and sometimes even revolutionary technologies, which pose a challenge to scientists to be further explored, exploited and applied in methodologies to determine trichothecenes. The electrochemical sensor technique as well as the transcriptomics technique and their practical applications have been introduced and discussed at leading international scientific conferences, such as the "IUPAC XIIth International Symposium on Mycotoxins and Phycotoxins", Istanbul, May 2007, and at the "World Mycotoxin Forum", November 2008. In addition, papers about both methodologies are published in "World Mycotoxin Journal" in 2009.

Training/Workshops

Currently the electrochemical immunosensor methods for DON and for T-2/HT-2 toxins are the most developed. Various demonstrations of electrochemical sensor techniques have been given, e.g. for the EU-National Reference Laboratories on mycotoxins in March 2009, and laboratory training is foreseen in 2010.

Figure 4: Demonstration activity for NRLs on Mycotoxins, March 2009

Future Activity

The necessary equipment for the electrochemical immunosensor methodology has been installed in various BioCop partner laboratories for further within- and between-laboratory testing. An extensive interlaboratory study is planned to be held in 2010, in close cooperation with the FP6 Network of Excellence project "MoniQA" and with various EU-National Reference Laboratories for Mycotoxins.

Also for the SPR methodology, the methods for DON and T-2/HT-2 toxins will be tested at partner laboratories and in inter-project cooperation with "MoniQA". For the transcriptomics methodology in-house validation of the detection system of T-2/HT-2 toxins is foreseen.

Frequently Asked Questions

Question:

? The electrochemical immunosensor methods are quite advanced. What are the advantages of the electrochemical immunosensor methods as compared to other screening techniques for trichothecenes?

Answer:

A The electrochemical immunosensor method makes use of low-cost screen-printed electrodes, and it is very is well suited for miniaturization and automation. It makes use of antibodies, specifically developed for BioCop, that cross-react equally with T-2 and HT-2 toxins, which is advantageous in view of expected regulations for the sum of these toxins.

Question:

? Various screening methods for mycotoxins currently exist. Is there really an interest for new mycotoxin screening methods as developed in BioCop?

Answer:

A An enquiry held at the BioCop workshop for the EU-National Reference Laboratories on Mycotoxins in March 2009 revealed that 44% of these NRLs currently makes use of screening methods to determine mycotoxins, and a much greater percentage (67%) of the NRLs indicated interest for screening methods as developed in BioCop.