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Application of proteomics and metabolomics for investigation of food toxins

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ABSTRACT

Fungi, bacteria and other organisms secrete into the extracellular environment numerous compounds that are required for their survival. The secreted proteins and the components of the translocation systems themselves can be scrutinized in-depth by the proteomic and other "omic" tools. Many of these secretion systems are involved in pathogenic processes and indicate mechanisms of pathogenesis and could as well be of great interest for the applications in food technology and biotechnology. Further improvements in existing and developing "omic" strategies and techniques will enable studies of fungal, plant and microorganism secretomes in order to build comprehensive and confident data sets of secreted proteins and other metabolites. Network of these components will lead to the increased understanding of interactions between the host and pathogen. The identification of proteins and small molecules that are produced by a still unknown pathogen will be the first step on the way of detection and further prevention of foodborne diseases.

This perspective brings the opportunity to develop new targets in order to ensure food safety that is important for human health as well as for the agriculture, food processing and storage. Ensuring food safety in the future will require new methods for identification, monitoring and assessing of foodborne hazards during production, storage, delivery and consume.

In present review the use of proteomics, peptidomics and metabolomics for determination of metabolic pathways and biomarkers of pathogenicity and resistance of biotoxins in the animal and plant food contamination is discussed.

1. Introduction

Food safety is the matter of global importance. Numerous outbreaks of food pathogens and toxins still occurred in both developing and industrialized countries. In addition, there has been considerable public interest in the safety of the investigation of fungal toxins, toxic chemicals in food and possible changes during the processing of foodstuffs (Käferstein & Abdussalam, 1999). Due to the changes in climatic conditions and environmental pollution, new toxic agents are identified and new toxic effects recognized. Therefore, the health and trade consequences of toxic chemicals in food also have global implications.

These events may impact contamination with mycotoxins in the soil, water and air, as well as production of seafood toxins, e.g. as a possible consequence of frequency and magnitude of oceanic harmful algal blooms (Marques, Nunes, Moore, & Strom, 2010). Poisoning caused by toxic components is sometimes difficult to link to a particular food; the onset of the effects may be gradual and not be detected until chronic or permanent damage occurs.

Surveillance studies showed that mycotoxin contamination is a world-wide problem, especially in developing countries, where suitable cultivation, processing and storage technologies are implemented with difficulty (Richard, 2007). In recent times, exposure to mycotoxins that can be found scattered in the environment has become a cause of growing concern. Soil, water, and air might be contaminated with outdoor environment from naturally occurring metabolites of fungal species that grow on a wide variety of crops or indoor environments from molds that can grow on various substrates. The identification of biomarkers of the most-relevant toxins that have been detected in this type of environment and their monitoring will yield a more accurate risk assessment (Bhatnagar et al., 2008).

Ensuring food safety is a responsibility among many participants in the food production chain such as manufacturer, distributor, regulatory and control agencies. One of the most important tasks of food safety is the elimination or at least the reduction of foodborne pathogens. These agents are a potential threat to human health and cause a variety of foodborne diseases. Additionally, the algal toxins from contaminated seafood can cause mostly acute disease. On the other hand, fungal toxins may be acutely toxic, but they may also have chronic sequelae, such as ateratogenic, immunotoxic, nephrotoxic, and estrogenic effects. The endemic nephropathy that occurs in Southeastern Europe is an example of chronical disease caused by an (still unknown) environmental

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agent. Of the many hypotheses put forward to disclose the causative agents, aristolochic acid originated in some food, possibly flour, or according to other interpretation, the mycotoxin ochratoxin A may be the principal risk factor for this chronic disease and endemic nephropathy associated urothelial-cancer (Arlt et al., 2007; Grollman & Jelaković, 2007; Huang et al., 2009; Pešić et al., 2011).

The consequences of climate changes (floods, droughts, strong winds, etc.) where the yield of food is reduced can cause the demographic changes as well as the change of the perception of food in developing countries. In industrial countries on the other hand, the new ways of the raw food processing will be directed into reduction and elimination of naturally occurring toxins. The continuing need for food safety puts increasing demands on food analysts to develop rapid and novel test methods. Thus, genomic, proteomic and metabolomic methods will have an important role in the food industry in order to ensure the production of safe and high-quality food in coming years (Gašo-Sokač, Kovač, & Josić, 2010).

Because of the ubiquity and complexity of identifying and monitoring, mycotoxins and seafood toxins are high on the list of candidates for the introduction of new methodologies, especially genomics, proteomics and metabolomics.

This paper presents an overview of the proteomic approaches in the investigation of food hazards such as toxic compounds in food.

2. Investigation of foodborne mycotoxins

Many strains of the molds, growing under favorable conditions, may produce metabolites, called mycotoxins that are toxic to humans and animals. Fungi infect crops and can also synthesize some toxins, which can be transmitted into the final food products (Zinedine, Soriano, Moltó, & Mañes, 2007).

Mycotoxins are considered secondary metabolites because they are not necessary for fungal growth and are products of primary metabolic processes. The functions of mycotoxins have not been clearly established, but they are believed to play a role in elimination of other microorganisms competing in the same environment.

Mycotoxigenic fungi are growing better under certain environmental conditions. The conditions in which these molds produce their mycotoxins are very specific and frequently independent of production of components that are required for fungal growth. The visible absence of mold does not mean that no mycotoxins are present since these may remain in the food long time after biosynthesis and secretion into the environment. *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* and *Claviceps* are the most common fungal species with the highest toxigenic potential. The determination of the mold flora in food is very important. Besides contamination, environmental conditions such as temperature and relative humidity as well as the type of structure of the food are favorable for the production of mycotoxins. It is not possible to entirely prevent the formation of mycotoxins, but eliminating conditions that enable the fungal growth helps to prevent their formation.

Mycotoxins can be found in a wide variety of matrices, ranging from cereals, peanuts, spices, animal feeds, fruits and vegetables and products of animal origin such as meat, milk and eggs. Around 400 mycotoxins have been registered until today (Richard, 2007). Twenty of them were found in food in amount which was sufficient to have a harmful effect on humans and animals. The classes of mycotoxins are aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids. Some of them are also documented as carcinogenic. Further mycotoxins with toxigenic potential are penitrem (a tremorgenic mycotoxin), patulin, cyclopiazonic acid and citrinin. Table 1 shows the main mycotoxins, mycotoxigenic fungi and their toxic effects.

Most mycotoxins do not decompose during the simple raw products processing and they remain in the final product even after the stringent production steps. The monitoring of these substances is necessary during whole production process, starting from the storage and transportation of the raw material until to the storage and consume of the final product.

The study of mycotoxins is very important due to their high level of toxicity as well as increasing legislative demands concerning their content in different products. National regulations and EU directives have been the main driver in the development of methods for detection and control of mycotoxins in the food production (van Egmond, Schothorst, & Jonker, 2007).

Analytical methods for mycotoxin determination can be divided into screening and confirmatory (reference) methods (Anklam, Stroka, & Boenke, 2002).

The screening methods include rapid protocols, among others, enzyme-linked immunosorbent analysis (ELISA), direct fluorimetry, fluorescence polarization, and various biosensors and strip methods, which are applicable for the determination of aflatoxins, fumonisins, ochratoxin A, zearalenone, and trichothecenes. Additionally, new

Table 1
Main mycotoxins in human food.
Taken from Capriotti et al. (2011) with permission.

Mycotoxins	Molds–toxins	Toxic effects
Aflatoxins	*Aspergillus flavus*, *A. parasiticus*, *A. nomius* Aflatoxins B1, B2, G1 and G2. Other aflatoxin metabolites M2, Q1, P1 and B1-8,9-epoxide	Hepatotoxic, immunosupressive, carcinogenic, teratogenic, mutagenic
Ochratoxin	*Aspergillus* and *Penicillium* ssp., *A. ochraceus*, *A. corbonarius*, *A. niger*, *P. verrucosum*, *P. Viridicatum* Ochratoxin A (OTA), ochratoxin B (OTB), ochratoxin C (OTC), 4-hydroxyochratoxin A and ochratoxin α.	Kidney and liver toxin, carcinogen; chronic toxicity as accumulates in the body
Trichothecenes	*Fusarium* spp. — *F. acuminatum*, *F. poae*, *F. sporotrichioides* Type A: T2, HT2, NEO, DAS, MAS *Fusarium* spp. — *F. graminearum*, *F. colmorum* Type B: DON, NIV, FUS-X, 3-ADON, 15-ADON	Vomiting, diarrhea, leukopenia, necrotic lesions, hemorrhage food refusal and vomiting, kidney problems, immunosupression
Fumonisins	*Fusarium verticillioides* (*moniliforme*), *F. proliferatum*, *F. subglutinans* Fumonisin B1 (FB1), fumonisins B2, B3 and B4.	Liver and kidney tumors, esophageal cancer, lung edema (swine), leukoencephalomalacia (horses)
Moniliformin	*F. proliferatum*, *F. subglutinans*	Acutely toxic, cardiac impairment
Ergot alkaloids: ergovaline, clavinet, Ergopeptines	*Claviceps neotyphodium*, *C. epichloe*, *C. purpurea*	Ergotism: gangrene, central nervous system symptoms (convulsions), gastrointestinal symptoms (vomiting)
ENN	*Fusarium beauveria*, *F. halosarpheia*, *F. paecilomyces*, *F. polyporus*, *F. verticillium* ENN: A, A1, B, B1; BEA	Acutely toxic, cardiac symptoms, herbicidal, insecticidal, antibiotic
Alternariol	*Alternaria* ssp. Alternariol (ALT), ALTOH, ALTMe, radicinin etc.	Acute toxicity
Patulin	*Penicillium*, *Aspergillus*, *Byssochlamys* ssp., *P. expansum*, *A. clavatus*, *B. fulva*, *B. Nivea* Patulin	Acutely toxic, toxic to kidneys; genotoxic, carcinogenic, teratogenic
Zearalenone	*Fusarium* spp. — *F. graminearum*, *F. colmorum*, *F. Equiseti*. ZEN, ZAN α- and β-ZOL	Estrogenic effects, reproductive toxicity

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