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Review

Recent advances in ochratoxin A-producing fungi detection based on PCR methods and ochratoxin A analysis in food matrices

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ABSTRACT

Ochratoxin A (OTA), a mycotoxin produced by various *Aspergillus* and *Penicillium* strains under diverse environmental conditions, has been found as a common contaminant of wide variety of cereals, dried fruits, spices, coffee and fermented beverages. Due to its widespread on such a large variety of agricultural commodities and the potential health risks, mainly toward humans, prompt detection is important.

To prevent OTA contamination in foodstuffs, recently several methods mostly based on PCR-based assays have been developed for identifying and quantifying OTA-producing fungi in food samples. PCR including its different formats remains the technique of choice, thanks to its ability to detect even small amounts of fungal DNA in raw materials and processed foods.

In order to meet food safety concerns and official legislated regulations, analytical techniques have been reported for OTA detection. Although most validated methods are chromatographic techniques, alternatives strategies are emerging and novel technologies using antibodies have been proposed, such as immunoassays, immunosensors and lateral-flow devices. Aptamers recently selected against OTA seem a promising tool and have been used these last years in bioanalytical methods for OTA detection, in electrochemical and optical techniques.

In this review, we discussed innovative analytical methods that have emerged in recent years for the detection of ochratoxigenic fungi and OTA in food samples.

Contents

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1. Introduction

Ochratoxin A (OTA) is a low molecular weight mycotoxin produced by certain strains of filamentous fungi of *Aspergillus and Penicillium* and detected in several food matrices. Among the OTA-producing fungi strains, most of them belong to two *Aspergillus* section: the section *Circumdati* (also called the *Aspergillus ochraceus* group) and the section *Nigri* (*Aspergillus carbonarius* and *Aspergillus niger*) (Varga, Kevei, Rinyu, Téren, & Kozakiewicz, 1996). Recently, *Aspergillus westerdijkiae* and *Aspergillus steynii*, two new species from *Aspergillus* section *Circumdati* have been split from *A. ochraceus* and reported to be stronger OTA producer than *A. ochraceus* (Gil-Serna, Vázquez, Sardiñas, González-Jaén, & Patiño, 2011). Among these species, *A. carbonarius* shows higher ochratoxigenic potential, and has been found to be responsible of OTA accumulation in grapes and wines, particularly in Mediterranean region, and in coffee and cocoa (Cabañes et al., 2002; Gallo et al., 2009; Serra, Abrunhosa, Kozakiewicz, & Venâncio, 2003). In contrast with the aspergilli, which occur mainly in regions with warmer climate, the penicillia prefer lower optimum growth temperature. Two *Penicillium* species, *Penicillium nordicum* and *Penicillium verrucosum* are known to produce OTA, and have been frequently isolated from cereal crops, meat products and cheeses (Bogs, Battilani, & Geisen, 2006; Lund & Frisvad, 2003).

Because of its widespread occurrence on a large variety of agricultural commodities and the potential health risks, mainly toward humans, OTA has been classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (Beardall & Miller, 1994). Given the known human exposure and the abundance of toxicological data from animal studies, the European Union Scientific Committee has recommended the OTA levels below to 5 ng/kg of body weight per day (Sweeney, White, & Dobson, 2000). In the European Union, some regulatory limits have already been introduced for the levels of OTA in food products such as raw cereal grains (5 μg/kg), products derived from cereals (3 μg/kg), dried fruits (10 μg/kg), roasted coffee and coffee products (5 μg/kg), grape juice (2 μg/kg) (EC No 123/2005) and also for all types of wine (2 μg/kg) (amended Regulation EC No. 466/2001).

A dual monitoring could be considered in order to meet food safety concerns and official legislated regulations. First, the presence of fungi having the potential to produce the OTA could be checked at critical points during production of agricultural commodities as well as during the process of food and feed preparation. Early detection of these fungi could prevent OTA contamination in foodstuffs, and protect consumers from hazardous mycotoxins. Usual identification and quantification methods of food-borne fungi require time-consuming and labor-intensive morphological and physiological tests and, often mycological expertise. Moreover, discrimination between the closely related species within the *Aspergillus* genus is particularly difficult, even for expert taxonomists (Dao, Mathieu, & Lebrihi, 2005; Mulè, Susca, Logrieco, Stea, & Visconti, 2006). The current trend is toward culture-independent PCR-based methods because they overcome problems associated with selective cultivation and isolation of microorganisms, and are generally characterized by their simplicity, speed, cost-effectiveness and reliability (Lau, Chen, Sleiman, & Sorrell, 2009; Niessen, 2008).

Subsequently, OTA could be detected directly in food sample using analytical methods able to perform highly selective measurements. Since OTA is a derivative of isocoumarinic acid linked to L-phenylalanine, this toxin displays an optical activity and fluorescence proprieties (Dall'Asta, Galaverna, Dossena, & Marchelli, 2004). For this reason, chromatographic techniques have been usually taken as reference methods because of their accuracy and reproducibility (See (Monaci & Palmisano, 2004; Turner, Subrahmanyam, & Piletsky, 2009) for a review). Nevertheless they are expensive and require long procedures and skilled staff, shortcomings that limit their use for routine analysis. Alternatively, biochemical methods and particularly the immuno-based assays appear as promising techniques for OTA detection. They usually require no or simple sample clean-up such as filtration and dilution, and allow parallel analysis of multiple samples, being a powerful tool for rapid screening (Turner et al., 2009). Recently aptamers have been shown to successfully compete with antibodies as biological receptors for analytical tool development. These single-stranded oligonucleotides are selected *in vitro* in a sort time compared to the antibody production, are more stable under a wide range of conditions. Furthermore, they can be easily modified or labeled providing flexibility to develop a wide range of assessment assays (Mairal et al., 2007).

The purpose of this review is to discuss the recently developed innovative analytical methods used in the analysis and detection of OTA-producing fungi and OTA itself in food matrices (Fig. 1).