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The Hidden Diversity of Potential Toxigenic Fungi from the Environment

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COLUMN ARTICLE

Diverse filamentous fungal species are able to synthesize mycotoxins, which are secondary metabolites that are not required for growth and survival, but instead can trigger toxic responses in animals and humans [1]. Relevant mycotoxin producing fungi related to the food chain can be found among members of the genera *Fusarium*, *Aspergillus* and *Penicillium* which can be present in environments as different as soils, plant- and animal-based foods and commodities [2-4]. From these, mycotoxigenic species of *Fusarium* spp. are considered among the most relevant toxigenic fungi present in agricultural soils as they can produce important and chemically diverse mycotoxins such as fumonisins. Other harmful mycotoxins of agro-food relevance are aflatoxins produced by members of *Aspergillus* spp. and also ochratoxins which are produced by members of both *Aspergillus* spp. and *Penicillium* spp. These mycotoxins have been evaluated for their carcinogenicity by the International Agency for Research on Cancer (IARC). As a result, aflatoxins were classified as carcinogenic to humans (Group 1) whereas fumonisins (including fumonisin B1 and B2) and ochratoxin A (OTA) were classified as possibly carcinogenic to humans (Group 2B) [5].

The identification of environmental fungi, including those present in agricultural soils, has been traditionally carried out through the cultivation of isolates and their subsequent characterization through different molecular and morphological techniques. A problem arises, however, due to a high proportion of fungi cannot be preserved in culture collections or cultivated on standard laboratory media under controlled laboratory conditions [6,7]. Therefore, the fungal diversity detected within an environment may be greatly biased and thus not be properly assessed. To circumvent this limitation culture independent approaches such as those based on metagenomics have been employed as they do not require the cultivation of microorganisms because they are based on the analysis of genomic DNA isolated from all the microbial community [8]. A collection of sequences obtained directly from environmental DNA, also referred to as metagenomic DNA, using the polymerase chain reaction (PCR) amplification and different sequencing methods, has been employed to study fungal diversity in different environments. It is worth noting that the number of different isolates obtained through cultivation and the number of different sequences from potential toxigenic fungi may overlap with each other [9]. As a result, both approaches could be used to obtain a more complete picture of the diversity of potential toxigenic fungi in a particular environment. To access this genetic diversity there are two main issues to be addressed and which can be summarized as 1) the DNA extraction method employed and 2) the genetic marker to recover enough phylogenetic signal.

In metagenomic studies, the isolation of high quality DNA

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is often a critical step. Environmental nucleic acids are frequently coextracted with humic acids and other substances which can lead to the inhibition of PCR reactions and other molecular procedures such as ligation and transformation during cloning processes [10]. The integrity of the DNA may be another important issue to take into account if a metagenomic library is intended to be constructed. The isolation of DNA from environmental fungi may be more challenging than in archaeal and bacterial microorganisms and, as a result, the DNA extraction method can affect the fungal diversity observed [11,12]. This is so because filamentous fungi are characterized by having a hard cell wall which may be difficult to break. Nevertheless, there are a number of DNA extraction protocols and commercial kits which employ mechanical forces and efficient chemical treatments to lyse the cells and thus isolate fungal DNA of acceptable quality and quantity for downstream applications [11-15]. Once the metagenomic DNA is conveniently extracted, it can be used for direct sequencing or for PCR amplification using different target sequences.

Another factor influencing the proper molecular identification of fungal isolates from the environment is the genetic marker of choice. It is better to use a variable region within the marker employed in order to obtain enough phylogenetic signal and specificity. A successful detection of fungal species has been carried out with primers designed from the nucleotide sequences of several genes such as the 18S and 28S rRNA genes, the elongation factor 1-α gene (EF-1α) and the β-tubulin gene, which can be suitable for identification purposes at the genus and species levels. More variable regions also used in identification are non-coding spacers such as the internal transcribed sequence (ITS), located between the 18S and 28S genes, and the nuclear ribosomal DNA intergenic spacer (IGS), which separates rDNA repeat units. From these, a better phylogenetic resolution is provided by the IGS region as this sequence represents the most rapidly evolving spacer region present within the rDNA array [16].

A significant progress has been made in the identification of toxigenic isolates belonging to different fungal species. For example, successful detection of variability among closely related *Fusarium* species and at the intraspecific level was achieved with genetic markers based on the IGS region and the EF-1α gene, which seem to provide the most phylogenetic signal [17-20]. However, it has not been until recent years that different members of *Fusarium* spp. have been detected in soil samples without cultivation by targeted sequencing [21-23]. Although the identification of potential toxigenic fungi from the environment can represent a challenge, it is necessary to emphasize the need of these studies as they will allow a better understanding not only of the genetic diversity of these filamentous fungi, but also of their distribution in a variety of habitats, including agricultural soils.

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