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PCR-based diagnosis and quantification of mycotoxin producing fungi

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Abstract

Mycotoxins are secondary metabolites produced by filamentous fungi which have toxicologically relevant effects on vertebrates when administered in small doses *via* a natural route. In order to improve food safety and to protect consumers from harmful contaminants, presence of fungi with the potential to produce such compounds must be checked at critical control points during production of agricultural commodities as well as during the process of food and feed preparation. Polymerase chain reaction (PCR) based diagnosis has been applied as an alternative assay replacing cumbersome and time consuming microbiological and chemical methods for detection and identification of the most serious toxin producers in the fungal genera *Fusarium*, *Aspergillus*, and *Penicillium*. The current review covers the numerous PCR-based assays which have been published over the last decade since the first description of the use of this technology to detect aflatoxin biosynthesis genes in *A. flavus*.
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1. Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi that in small concentrations can evoke an acute or chronic disease in vertebrate animals when introduced *via* a natural route (Gravesen et al., 1994). Some compounds which are usually subsumarized among the mycotoxins do not exactly fit the above definition, *e.g.* zearalenone, which acts as an estrogen analogue (Mirocha et al., 1978). The majority of the >400 mycotoxins currently known (Bauer and Gareis, 1987) are grouped according to their toxic activity under chronic conditions as mutagenic, carcinogenic or teratogenic. Grouping according to their site of action results in hemo-, hepato-, nephro-, dermato-, neuro- or immunotoxins. Exposure may occur through ingestion, inhalation or dermal contact (Pitt, 2000).

Mycotoxins are among the oldest environmental toxicants menacing human existence since ancient times (Kampelmacher, 1973). To date, massive outbreaks of human mycotoxicoses occur but are more or less restricted to developing countries, *i.e.* outbreak of acute aflatoxicosis in the Makueni district and neighbouring districts in Kenya in 2004 with 125 fatalities as the most recent case (Muture and Ogana, 2005). In developed countries and in threshold countries the major concerns are chronic effects of ingesting small concentrations of mycotoxins over a long period of time (Etzel, 2002). The majority of mycotoxin producers can be found in the fungal genera *Aspergillus, Penicillium, Fusarium* and *Alternaria* which concomitantly happen to be the most abundant contaminants of food and feed. All four genera have in common the presence of high numbers of species, distinction of which needs a high degree of specialization. Moreover, constant changes in the taxonomy of these genera may lead to misidentification of an isolate and false evaluation of its toxigenic potential.

Aimed at both overcoming the obstacles of identification as well as developing rapid tools for detection, nucleic acid based methods have been developed and used recently as a tool for the analysis of mycotoxigenic fungi. The polymerase chain reaction (PCR, Saiki et al., 1988) has replaced cumbersome and time consuming microbiological analysis by amplification of specific genomic markers rather than growing the living organism under study. Within the 10 year period of 1996–2006 PCR-based detection systems have been set up for the major species and groups of mycotoxigenic fungi. Systems were developed in three major "waves" of innovation which very much reflect the three periods in which different groups of toxins came into focus of scientific interest and public

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awareness. Fig. 1 gives a graphic representation of publications over time from 1996 through 2006. It must be noted here that only the first publication of a detection system for the depicted species and groups of species are shown. In many cases alternative PCR-based systems have also been described later.

The following provides an overview of the use of PCR-based systems available to date for molecular identification and detection of producers of aflatoxins, trichothecenes, fumonisins, and patulin. Systems for detection of ochratoxin A producing fungi have been recently reviewed elsewhere (Niessen, 2007) and will not be considered further in this review.

2. Detection of aflatoxin producers

The first PCR-based systems were developed for producers of aflatoxins as the most potent compounds among the mycotoxins. The first PCR-based detection system for a mycotoxin producing fungus was published by Tang et al. (1993) who described detection of *A. flavus* by nested PCR in human bronchoalveolar lavages. Their primers were based on the gene coding for alkaline protease. However, this early publication was aimed at *A. flavus* as a lung pathogen rather than a toxin producer. Clearly focussed on evaluation of toxigenic properties of *Aspergillus* species from section *Flavi* were two PCR assays published by Geisen (1996) and a parallel publication by Shapira et al. (1996). Both papers can be regarded as the starting point for PCR-based diagnosis of mycotoxigenic fungi. Authors used sequences of the same three genes involved in the biosynthesis of aflatoxins in *A. flavus, A. parasiticus*, and *A. versicolor* to design their specific primers. The assay published by Geisen (1996) made use of the three primer pairs in a multiplex PCR in which it was demonstrated that *A. sojae* and *A. oryzae*, both of which are essentially identical with *A. flavus* but typically do not produce aflatoxins, lack the *nor-1* gene. Other, non aflatoxin producing *A. flavus* strains also gave no rise to a PCR product with one or all of the primer pairs used. Feasibility of both co-published assays for detection of their target in contaminated corn (Shapira et al., 1996), cereals (Geisen et al., 1998), as well as figs (Färber et al., 1997) was demonstrated in follow up studies. Recently, Mayer et al. (2003) used sequences of the *nor-1* gene to set up primers and a probe for a TaqMan™ real-time PCR assay with which *A. flavus* was quantified in contaminated food samples and in cereals. Using a different concept for primer design and SYBR-Green I as fluorescent dye, Bu et al. (2005) described a quantitative real-time PCR assay for *A. flavus*, among other medically important fungi, in pure cultures and in medical specimens. Primers used were based on sequences from the ITS1-5.8 S region of the rRNA gene of *A. flavus*. Similar to the formerly mentioned work, the conventional PCR assay described by Sugita et al. (2004) made use of specific primers designed from the sequence of another part of the ITS1 region of the *A. flavus* rRNA gene to analyse medical samples. Besides the *Aspergillus* spp. mentioned above, *A. nomius* is another species in section *Flavi* which is known as a producer of aflatoxins but also of tenuazonic acid (Frisvad and Thrane, 2004). Haugland and Vesper (2000) published primers for the diagnosis of a wide range of fungal species in a patent application. The system described for specific detection of *A. nomius* was based on sequences from the ITS region of the rRNA gene and was described together with a fluorescently labelled probe to be applied in a TaqMan™ quantitative real-time PCR assay. No PCR-based detection assays have been described yet for the rarely encountered aflatoxin producers *A. bombycis, A. ochraceoroseus* and *A. pseudotamari* (Bennett and Klich, 2003).

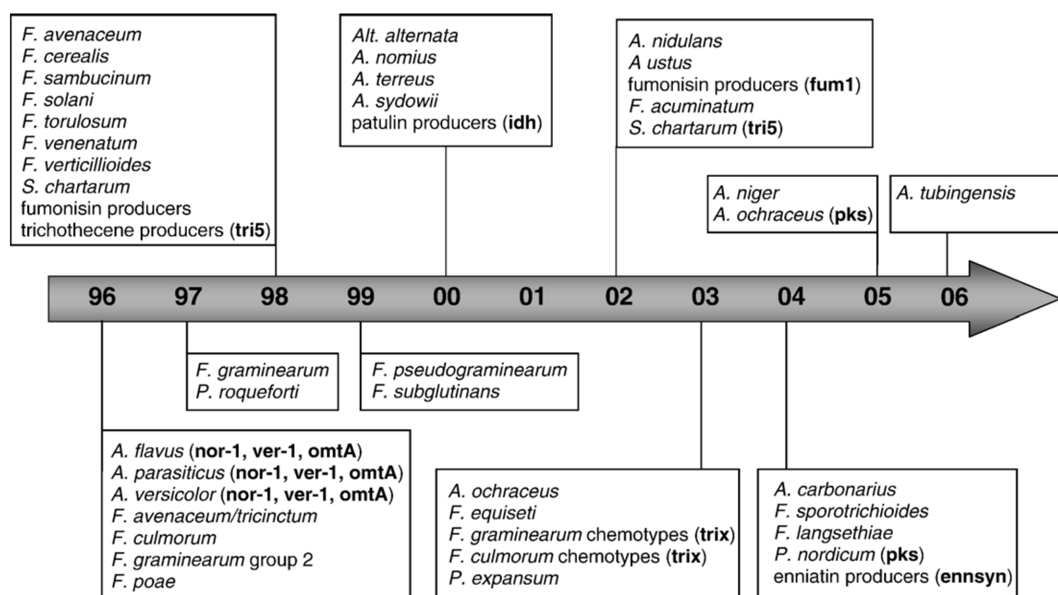

Fig. 1. Development of PCR-based detection systems for mycotoxin producing fungi from 1996 until present. First publication of a diagnostic primer pair was taken as time mark for each species depicted. In many cases, more systems were published later for the same fungus. Mycotoxin biosynthesis genes used as sequence source are given in bold.

3. Detection of trichothecene producers

Trichothecenes are sesquiterpenoid mycotoxins which share the 12,13-epoxytrichothecene skeleton as the common structural feature. Presence or absence of an 8-keto moiety leads to differentiation of group B and group A trichothecenes, respectively, the latter of which have a valerianyl-, acetyl-, or hydroxyl moiety in that position. Furthermore, group C trichothecenes (macrocyclic trichothecenes) are d0ifferentiated by the presence of a macrolide ring system attached at position 4β and 15 of the trichothecene verrucarol (Grove, 1993). Trichothecenes are produced by species in the fungal genera *Cryptomela, Fusarium, Myrothecium, Stachybotrys, Trichoderma/Hypocrea, Trichothecium* and *Verticimonosporium* (Turner and Aldridge, 1983; Davis and Diener, 1987; Frisvad and Thrane, 2004). Toxins of the trichothecene type were also found to be produced by a hypocrealean epibiont of the plant species *Baccharis cordifolia* (Jarvis et al., 1991; Rosso et al., 2000). *Fusarium* spp., however, produce the widest variety of different trichothecene compounds among which the type B-trichothecenes deoxynivalenol (DON) and nivalenol (NIV) as well as the type A-trichothecenes T-2 toxin, HT-2 toxin, neosolaniol (NEOS), and Diacetoxyscirpenol (DAS) are the most common and/or toxic compounds isolated from natural sources.

The genetics and regulation of trichothecene biosynthesis have been elucidated in much detail in *F. sporotrichioides* (Hohn et al., 1993), *Myrothecium roridum* (Trapp et al., 1998), and *F. graminearum* (*Gibberella zeae*, Kimura et al., 2003). Sequencing of parts of the trichothecene gene cluster was done for species in the *F. graminearum* group, *i.e. F. crookwellense, F. culmorum, F. lunulosporum* and *F. pseudograminearum*. The *tri5* gene, which codes for trichodiene synthase catalysing the first specific step in the biosynthesis of all producing fungi was particularly well characterized in *Fusarium* spp., and in *Stachybotrys chartarum* (Strauss and Wong, 1998 direct submission to GenBank), *Myrothecium roridum* (Trapp et al., 1998), and *Trichoderma harzianum* (Gallo, 2004). Niessen and Vogel (1998) aligned *tri5* gene sequences of several *Fusarium* spp. to find two highly conserved regions within the gene. Primers designed for those regions amplified a 658 bp product from 20 different species and varieties within *Fusarium*. Based on the same gene, Schnerr et al. (2001) designed primers which resulted in a smaller PCR product detecting essentially the same set of species as in Niessen and Vogel (1998). A quantitative real-time PCR (SYBR green I) was set up to quantify DNA concentrations of trichothecene producers in 300 naturally infected samples of wheat. Comparison of DNA- and DON concentrations in the samples revealed a positive correlation between both parameters. Primers developed by Niessen and Vogel (1998) were applied in follow up studies by various authors to detect the *tri5* gene in pure cultures and in sample materials by conventional PCR (Tan and Niessen, 2003; Agodi et al., 2005; Demeke et al., 2005). Sequence information was also used to set up species specific assays for identification, detection, and quantification of typical trichothecene producers among fusaria. During a project in which a polyphasic approach was applied to study the taxonomy of species within the section *Sporotrichiella* of *Fusarium*, Niessen et al. (2004) developed *tri5* gene based primers to set up species specific detection assays for the newly described *F. langsethiae* (Torp and Nirenberg, 2004) and for *F. kyushuense, F. poae* and *F. sporotrichioides*. The detection systems made use of the forward primer described in Niessen and Vogel (1998) which was combined with reverse primers binding specifically to the intron region of the *tri5* gene in the respective species. Using the same gene, Strausbaugh et al. (2005) recently set up a TaqMan™ quantitative real-time PCR assay for quantification of *F. culmorum* in wheat roots and in barley plants.

Stachybotrys chartarum (syn. *S. atra*) was identified as a producer of the highly potent macrocyclic trichothecene satratoxin in “sick building syndrome” households (Etzel et al., 1998). Strauss and Wong published the DNA sequence of the *tri5* gene of that fungus (GenBank, direct submission (1998), accession no.AF053926). The sequence was subsequently used by Peltola et al. (2002) to design a pair of specific PCR primers for the identification of the toxigenic subgroup 1 of *S. chartarum*. Non toxic strains and strains with low toxicity were combined in subgroup 2. These fungi did not give a product with the primer pair which thus was useful to distinguish the two taxonomical groups in *S. chartarum*. Presence of two lineages in this fungus was also found by Koster et al. (2003), who described two phylogenetically distinct lineages when testing genotypic variation of *tri5*, ITS and two housekeeping genes in a geographically diverse set of isolates. Dean et al. (2005) developed another pair of *tri5*-based primers for identification of *S. chartarum* which were combined with primers specific for *A. versicolor, P. purpurogenum,* and *Cladosporium* spp., respectively, in a multiplex PCR assay to check buildings for the potential presence of mycotoxin producing fungi in indoor environments.

Besides *tri5*, other genes from the trichothecene biosynthesis cluster were used to design species- and group specific PCR primers. A group specific PCR assay for the detection of trichothecene producing *Fusarium* spp. involving primers binding to the *tri6* gene (transcription factor), was set up by Bluhm et al. (2002). The authors used the system together with primers for sensitive detection of fumonisin producers. Bakan et al. (2002) applied intergenic sequences between the *tri5* and *tri6* genes to distinguish between high and low DON producing *F. culmorum* isolates. Testing of 17 and 13 isolates of high and low DON producers, respectively, revealed 100% correlation between producer type and the size of amplicon produced. In order to differentiate DON and NIV producing chemotypes in *F. graminearum*, Lee et al. (2001) designed primers hybridising adjacent to an inserted region present in the *tri7* gene of the fungus. Chemotypes were characterized by amplicons of 161 bp and 173–327 bp for NIV and DON producers, respectively. A genus specific primer pair hybridising to sequences within the *tri13* gene was published by Demeke et al. (2005), who screened 85 samples of wheat, barley, oats and corn from Canada and found good correlation between PCR detection of *F. graminearum* biomass and presence of DON in the samples.

Various other sequence sources other than genes from the trichothecene biosynthesis pathway were used to set up PCR-based diagnostics for trichothecene producers. Highly specific identification of *F. graminearum* was demonstrated by Niessen and Vogel (1997) who used the galactose oxidase (*gaoA*) gene as sequence source to set up a species specific PCR assay. Using the same primer pair in a triplex PCR assay, Knoll et al. (2000) demonstrated the usefulness of the method for detection of trichothecene producers as well as *F. culmorum* and *F. graminearum* in contaminated wheat. The same primers were also used by Knoll et al. (2002) in an assay involving DNA Detection Test Strips™ and a specific probe for rapid detection of the PCR product. Another alternative detection and quantification method was used by Niessen et al. (1998), who employed the *gaoA* specific primers in a solid phase PCR assay using the DIAPOPS technology (Chevrier et al., 1993).

Two different housekeeping genes were used as the sequence source for the development of PCR identification and detection systems for trichothecene producing *Fusarium* spp. The gene coding for elongation factor 1 α (EF-1 α) was used by Aoki and O´Donnell (1999) as sequence source for a PCR assay which detected *F. pseudograminearum* with high precision. In another study, Reischer et al. (2004) aligned sequences of the beta tubulin gene from several isolates of *F. graminearum* and designed a primer pair for the specific identification of that fungus in a TaqMan™ quantitative real-time PCR assay to quantify biomass of the fungus in field inoculated wheat plants.

Ribosomal RNA gene sequences were utilized by various authors to design specific PCR primers. Most authors used variable regions present within the internal transcribed spacers separating the genes coding for 18S rRNA and 5.8S rRNA (ITS1) and between the latter gene and the gene coding for 28S rRNA (ITS2). Haugland and Vesper (2000) filed a patent with the US Patent and Trademark Office in which detection of a wide variety of fungal organisms based on rRNA sequences was claimed using the TaqMan™ technology for sensitive detection in real-time PCR. Mishra et al. (2003) used the ITS region in the rRNA genes of *F. avenaceum, F. culmorum, F. equiseti, F. oxysporum* and *F. sambucinum* to identify the target organisms in a four color fluorescent assay. An assay published by Kulik et al. (2004) was based on the ITS2 region of the rRNA gene. The assay was useful for identification of *F. sporotrichioides* pure cultures but also for detection of the fungus in plant tissue.

As an alternative to the use of sequence information from defined genes or otherwise functionally defined DNA, sources of undefined origin have been used to set up specific PCR primers. Such undefined sequence sources can be generated by random amplification of genomic DNA (RAPD) with short (decamer) oligonucleotide primers under quite unspecific conditions (Williams et al., 1990). Patterns may show polymorphic markers, the DNA sequence of which has been used as source for the design of specific PCR primers for *Fusarium* spp. early during the development of PCR as a diagnostic tool in toxigenic fungi. Schilling et al. (1996) were among the first authors to report on the use of a PCR-based detection systems for *Fusarium* species. Their primers for identification of *F. culmorum, F. graminearum,* and *F. avenaceum* were used to detect the three species in contaminated cereals. Primers developed by Schilling et al. (1996) were used in various follow up studies by other authors who found further proof for their usefulness (Chelkowski et al., 1999; Gargouri et al., 2001; Obradovic et al., 2001; Williams et al., 2002; Klemsdal and Elen, 2006). Nicholson et al. (1998) used RAPD based primers to set up competitive quantitative PCR assays for *F. graminearum* and *F. culmorum* in which internal competitor fragments added in defined concentrations compete for primer binding with the target DNA, thus enabling quantification. Yoder and Christianson (1998) applied RAPD to the taxonomic study of species within section *Discolor* of *Fusarium*. Taxon specific RAPD markers were obtained and PCR identification systems were set up for *F. crookwellense, F. culmorum, F. graminearum, F sambucinum, F. torulosum* and *F. venenatum*, to which species the fungus used in the production of mycoprotein (Quorn), was finally assigned as a result of that study.

4. Detection of fumonisin producers

Fumonisins are a group of mycotoxins produced by species within the *Gibberella fujikuroi* complex of species. The major producers are *F. verticillioides, F. proliferatum, F. subglutinans* and *F. nygamai* (Bennett and Klich, 2003). They affect animals by interfering with their sphingolipid metabolism (Merrill et al., 2001). Besides various animal diseases they are suspected to be responsible for certain forms of human esophageal cancer observed in Transkai (South Africa), China, and Northeast Italy (Sydenham et al., 1991; Peraica et al., 1999). Moreover, fumonisin B_1 has been associated with neural tube defects in experimental animals and may therefore be involved in cases of *spina bifida* in humans (Hendricks, 1999). The toxin has been assigned a 2B carcinogen (probably carcinogenic to humans) status by the International Research Agency for Cancer (IARC, 1993; Rheeder et al., 2002). As in other mycotoxin producers, genes involved in the biosynthesis and regulation of fumonisins are organized in clusters within the genome. Waalwijk et al. (2004a) studied the fumonisin gene cluster of *F. proliferatum* and found 19 genes to be involved in biosynthesis and regulation of the toxin.

F. proliferatum (mating population D of *Gibberella fujikuroi*, Leslie, 1995) was isolated from rice, corn, and other cereals in tropical and subtropical countries (Samson et al., 2004). Beck and Barnett (2003) filed a patent to the US Patent and Trademark Office, in which primer pairs for the specific identification of *F. proliferatum* and *F. verticillioides* were described. The system was based on the sequence of the small subunit of the mitochondrial rRNA gene. Using a partial sequence of the calmodulin gene from *F. proliferatum, F. subglutinans,* and *F. verticillioides*, Mulé et al. (2004) designed specific primer pairs for the identification of the three species. Analysis of DNA from 150 strains revealed that the primers were highly specific for their respective target species. Möller et al. (1999) used RAPD based markers as sequence source for the development of PCR-based detection systems for *F. verticillioidese* and *F. subglutinans,* which were analyzed in maize in a multiplex PCR where primers

for detection of both species were combined. Zheng and Ploetz (2002) developed another RAPD-based PCR which they applied to the screening of *F. subglutinans* isolates from *Mangifera indica* where the fungus causes a destructive disease called mango malformation. Testing various isolates of different species within the *G. fujikuroi* complex showed that the system was specific for *F. subglutinans* and *F. nygamai*. DNA from the latter species resulted in amplification of a significantly shorter product with the primer pair developed by Zheng and Ploetz (2002). Another approach using genomic markers of unknown function was applied by Murillo et al. (1998) who used the sequence of shotgun fragments of genomic *F. verticillioides* DNA to set up a primer pair which enabled amplification of a 1600 pb PCR product with DNA of that species. However, in a later study Möller et al. (1999) tested the primers developed by Murillo et al. (1998) with DNA from other species from the *G. fujikuroi* complex and found cross reactivity with isolates of mating populations B, C, D, E, F and also with *F. nygamai* and *F. oxysporum*.

Attempts were made to use PCR to study the mycotoxigenic potential of fumonisin producers because it is well established that only a certain percentage of isolates are able to produce the toxin *in vitro* and *in planta*. Based on sequence variation within the intergenic region (IGS) of the rRNA gene between fumonisin producing and non producing isolates of *F. verticillioides*, Patino et al. (2004) set up a highly sensitive PCR system for toxigenic isolates among 54 strains of *F. verticillioides* from different geographical regions and hosts. Grimm and Geisen (1998) compared nucleotide sequences of the ITS1 region within the rRNA genes of typical fumonisin producing *Fusarium* species with those of species that do not typically produce that toxin. A pair of PCR primers and a biotinylated probe were designed which made use of sequence differences to specifically amplify a 108 bp fragment from fumonisin producers. For detection of the PCR fragment, Grimm and Geisen (1998) used a microplate-based enzyme-linked oligosorbent assay (ELOSA) with a biotinylated capture probe and detection of bound PCR fragments *via* binding of enzyme linked anti-DIG antibodies to DIG incorporated into the PCR-product. The assay was specific for detection of fumonisin producing isolates of *F. verticillioides, F. proliferatum, F. nygamai* and *F. napiforme*.

Recently, two PCR-based assays were published which used sequence information from genes involved in the biosynthesis of fumonisin for the selective identification of toxigenic isolates of *F. verticillioides*. Gonzalez-Jaen et al. (2004) demonstrated that genes *fum1* (=*fum5*), *fum6,* and *fum8* were only present in *F. verticillioides, F. proliferatum, F. fujikuroi* and *F. nygamai*, which represent the principle producers of fumonisins within the *G. fujikuroi* complex. Primers were derived from the sequence representing the β-ketoacyl reductase domain within the *fum1* gene. Primers appeared to be highly specific for *F. verticillioides* isolates which produced fumonisin *in vitro* and it was assumed that the non producing isolates must have lost the *fum1* gene or at least the part of it where PCR primers hybridise in fumonisin producers. Recently, Sanchez-Rangel et al. (2005) reported similar results with a different pair of primers for the same part of the *fum1* gene. In contrast to Gonzalez-Jaen et al. (2004), correlation of PCR results with *in vitro* fumonisin production of *F. verticillioides* revealed a number of cases in which a *fum1* gene was detected but toxin concentrations produced were negligible or very low. It was speculated that the principle ability of a *F. verticillioides* isolate to produce fumonisin will depend on the presence or absence of the *fum1* gene but additional factors may be necessary which regulate the concentrations of fumonisin finally produced. Another group specific assay for fumonisin producers was published by Bluhm et al. (2002). The system was based on the *fum1* gene sequences of *F. proliferatum* and *F. verticillioides* and was applied to the detection of these fungi in artificially contaminated cornmeal in a multiplex PCR assay. Potential producers of fumonisins were detected together with potential trichothecene producers (*tri6* gene).

5. Detection of patulin producers

Patulin is a tetraketide mycotoxin produced by a variety of different fungi, most of which are referable to the ascomycete genera *Byssochlamys* and *Eupenicillium*. Frisvad and Filtenborg (1989) found *P. carneum, Paecilomyces variotii* and *P. glandicola* to be the main producers in silage, whereas *P. coprobium, P. glandicola, P. vulpinum, P. clavigerum* and *P. concentricum* are the main producers among coprophilic fungi. Also *Aspergillus* species (*A. clavatus, A. giganteus, A. terreus*) were found to be effective producers of the toxin. In foods, however, *P. expansum* and *P. griseofulvum* are the major producers of patulin with apples and unfermented apple juice being the main source of the toxin in human consumption. JECFA has established a provisional maximum tolerable daily intake for the compound of 0.4 mg/kg bw per day. Patulin is regulated in the European Union at levels of 50, 25 and 10 μg/kg in fruit juices and fruit nectar, solid apple products and apple-based products for infants and young children, respectively (Regulation (EC) No 466/2001, amended by Regulation (EC) No 455/2004).

The gene coding for Isoepoxidon dehydrogenase (*idh*) is a key gene in patulin biosynthesis (Sekiguchi and Gaucher, 1979). Using the *idh* gene sequence (Fedeshko, 1992), Paterson et al. (2000) set up a gene specific PCR for producers of patulin. Testing of various isolates of *P. expansum* revealed general ability of patulin production *in vitro* and presence of the *idh* gene in this species. Also in *P. brevicompactum*, most isolates produced the toxin but few obviously had the *idh* gene present but were unable to produce patulin *in vitro*. Authors demonstrated the usefulness of their primer pair to detect the gene in contaminated twigs, bark, soil and fallen apples but failed to have a positive reaction in healthy apples picked just prior to analysis. In a follow up study, Paterson (2004) demonstrated that the *idh* gene is a quite wide spread feature in the genus *Penicillium* but he also showed presence of the gene in *Aspergillus*, *Paecilomyces*, and *Byssochlamys*. Recently, Paterson (2006) demonstrated usefulness of *idh* specific primers for the analysis of orchard soils as critical control point in a HACCP concept for prevention of patulin contamination in apple products.

Based on the nucleotide sequence of the polygalacturonase gene of patulin producing *P. expansum*, Marek et al. (2003) designed a pair of primers for specific identification and sensitive detection of that fungus in pure cultures. *P. carneum* and *Paecilomyces variotii* are producers of patulin which frequently occur in silage and may therefore be dangerous as a contaminant of animal feed. Pedersen et al. (1997) designed primers binding to the ITS region of *P. roqueforti* and *P. carneum* isolates so that both species were detected in complex food samples (cereals, cheese).

6. Conclusions and future perspectives

The present literature review has revealed a tremendous toolbox of oligonucleotide primers developed during the decade 1996–2006. Diagnostic PCR-based systems are now available for all of the relevant toxigenic fungi, and for many of the less relevant species, as well. In many publications specificity has been tested with a more of less extensive set of fungal species. However, some of the publications reviewed here do not offer much detail regarding the spectrum of species picked up by a primer pair. It should therefore become standard procedure in future developments to test cross reactivity of a primer pair at least with the most closely related species. Building up a new assay should start with an *in silico* analysis of possible cross reactions with the primers designed. This first check should give a gross overview over the spectrum of species which are to be tested for cross reaction. Testing of a wide taxonomical spectrum becomes even more demanding if the target sequence for primer design was taken from genes coding for universal proteins, *e.g.* TEF1α, β-tubulin, calmodulin or ribosomal RNA genes. The spectrum of species tested should also take into account the funga potentially occurring in sample materials planned to be analyzed with the future assay and the DNA isolated from the sample itself if food or feed material is involved. Various PCR-based assays developed during the last decade used genes as sequence sources for primer design which were either present only in the target species but unrelated to mycotoxin biosynthesis, *e.g. gaoA* in *F. graminearum* (Niessen and Vogel, 1997), or which were involved in the biosynthesis and regulation of the mycotoxins produced. Examples for assays developed along that line may be found for all of the mycotoxins of major concern, except for producers of zearalenone. Biosynthesis of that toxin is currently under investigation and various genes involved have recently been characterized (Gaffoor and Trail, 2006; Lysoe et al., 2006). Development of PCR-based detection systems based on those genes can therefore be anticipated in due course.

One of the major motivations for the development of PCR-based detection systems in many publications is the prospect of using this kind of analysis to forecast the presence and concentrations of mycotoxins in sample material. One might therefore anticipate that assays based on mycotoxin biosynthetic genes might better fit that purpose as compared to systems based on genes unrelated to their biosynthesis. However, only in a few cases correlation between presence of a PCR signal or its intensity and incidence or concentrations of certain mycotoxins have been analysed, and in even fewer cases have positive correlations been established. At this point, the general question has to be raised, is PCR with genomic target DNA as template the technology of choice to estimate mycotoxin concentrations in sample materials? Given the fact that biosynthesis of most mycotoxins is a highly complex process with poorly understood regulation at the transcriptional level (other mechanisms as well?) as well as it is highly influenced by environmental factors, it seems unlikely to find a highly positive correlation between amplification signal and concentrations of compounds. Study of the literature shows that in few cases such a correlation has been established with quantitative real-time PCR on mycotoxin biosynthesis genes (Schnerr et al., 2002; Sarlin et al., 2006). However, similarly good correlations between results of quantitative real-time PCR and mycotoxin concentrations were found when primers were targeted to anonymous sequences (Waalwijk et al., 2004b; Leisova et al., 2006) or to sequences of housekeeping genes (Mulé et al., 2006). Other authors found very poor correlation between the parameters (Schmidt et al., 2004). Overall, this means that prediction of mycotoxin concentrations using a PCR-based system is unlikely to work in a way that would allow for this technique to replace analysis of mycotoxins unless assays are developed which are based on expression of genes involved in mycotoxin biosynthesis, *i.e.* systems using cDNA as target for amplification. Such a system has recently been developed by R. Geisen's group at the Federal Institute for Nutrition in Karlsruhe, Germany. The system makes use of a microarray to which cDNA of genes involved in the production of OTA can be immobilized and visualized by fluorogenic detection probes. Good correlations of signals were found to fungal biomass but also to OTA produced under various environmental conditions.

With the currently available systems for PCR-based detection and identification, however, qualitative information about the presence or absence of a certain fungus can be obtained and this should be used to advantage for food and feed quality control because the technology has the power to provide an insight into the mycotoxigenic potential of a sample analyzed. This information can than be used in order to decide whether a lot should go further down the process of production or should be retained for further analysis of mycotoxins. Which toxins to analyze could be figured out with PCR-based multiplex systems as recently introduced by Kristensen et al. (2006a,b) who used a DNA microarray and the SnaPshot technology to detect and differentiate 16 different mycotoxin producing *Fusarium* spp. in a single multiplex assay. This platform offers the possibility of detecting very high numbers of different fungal species and groups of species in short time with relatively low labour input.

The systems described above might show one possible way in which molecular detection of mycotoxigenic fungi may be analyzed and controlled in order to optimize food and feed production processes for minimized risk of mycotoxin production. In analogy to computer aided manufacturing (CAM) this would be addressed as genome aided processing (GAP) in food production. Still, enormous work has to be done in order to accomplish that state. Keeping in mind, however, that most of

the developments described in the current review were done in very recent years, GAP based systems may become reality within a short time and be applied for the health and welfare of consumers.

References

Agodi, A., Barchitta, M., Ferrante, M., Sciacca, S., Niessen, L., 2005. Detection of trichothecene producing *Fusarium* spp. by PCR: Adaptation, validation and application to fast food. Ital. J. Public Health 3, 7–11.

Aoki, T., O´Donnell, K., 1999. Morphological and molecular characterization of *Fusarium pseudog raminaearum* sp. nov., formerly recognized as the group 1 population of *F. graminearum*. Mycologia 91, 597–609.

Bakan, B., Giraud-Delville, C., Pinson, L., Richard-Molard, D., Fournier, E., Bryggo, Y., 2002. Identification by PCR of *Fusarium culmorum* strains producing large and small amounts of deoxynivalenol. Appl. Environ. Microbiol. 68, 5472–5479.

Bauer, J., Gareis, M., 1987. Ochratoxin A in der Nahrungsmittelkette. J. Vet. Med., B 34, 613–627.

Beck, J.J., Barnett, C.J., 2003. Detection of *Fusarium* species infecting corn using the polymerase chain reaction. U.S. Patent Application #200330113722, June 19, 2003, 23 pp.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497–516.

Bluhm, B.H., Flaherty, J.E., Cousin, M.A., Woloshuk, C.P., 2002. Multiplex polymerase chain reaction assay for the differential detection of trichothecene- and fumonisin-producing species of *Fusarium* in cornmeal. J. Food Prot. 65, 1955–1961.

Bu, R., Sathiapalan, R.K., Ibrahim, M.M., Al-Mohsen, I., Almodavar, E., Gutierrez, M.I., Bhatia, K., 2005. Monochrome lightcycler PCR assay for detection and quantification of five common species of *Candida* and *Aspergillus*. J. Med. Microbiol. 54, 243–248.

Chelkowski, J., Bateman, G.L., Mirocha, C.J., 1999. Identification of toxigenic *Fusarium* species using PCR assays. J. Phytopathol. (Berlin) 147, 307–311.

Chevrier, D., Rasmussen, S.R., Gues-Jon, J.L., 1993. PCR product quantification by non radioactive hybridization procedures using an oligonucleotide covalently bount to microwells. Mol. Cell. Probes 7, 187–197.

Davis, N.D., Diener, U.L., 1987. Mycotixins. In: Beuchat, L.R. (Ed.), Food and Beverage Mycology. Avi, New York, pp. 517–570.

Dean, T.R., Roop, B., Betancourt, D., Menetrez, M.Y., 2005. A simple multiplex polymerase chain reaction assay for the identification of four environmentally relevant fungal contaminants. J. Microbiol. Methods 61, 9–16.

Demeke, T., Clear, R.M., Patrick, S.K., Gaba, D., 2005. Species specific PCR-based assays for the detection of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis. Int. J. Food Microbiol. 103, 271–284.

Etzel, R., 2002. Mycotoxins. J. Am. Med. Assoc. 287, 425–427.

Etzel, R.A., Montana, E., Sorensen, W.G., Kullman, G.J., Allan, T.M., Dearborn, D.G., 1998. Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. Arch. Pediatr. Adolesc. Med. 152, 757–762.

Färber, P., Geisen, R., Holzapfel, W.H., 1997. Detection of aflatoxinogenic fungi in figs by a PCR reaction. Int. J. Food Microbiol. 36, 215–220.

Fedeshko, R.W.,1992. Polyketide Enzymes and Genes, 2 vols. PhD thesis, University of Calgary.

Frisvad, J.C., Filtenborg, O., 1989. *Terverticillate penicillia*: chemotaxonomy and mycotoxin production. Mycologia 81, 837–861.

Frisvad, J.C., Thrane, U., 2004. Mycotoxin production by common filamentous fungi. In: Samson, R.A., Hoekstra, E.S., Frisvad, J.C. (Eds.), Introduction to Food- and Airborne Fungi. Centraalbureau voor Schimmelcultures, Utrecht, pp. 321–330.

Gaffoor, I., Trail, F., 2006. Characterization of two polyketide synthase genes involved in zearalenone biosynthesis in *Gibberella zeae*. Appl. Environ. Microbiol. 72, 1793–1799.

Gallo, A., 2004. Isolation and characterisation of a trichodiene synthase homologous gene in *Trichoderma harzianum*. Phys. Mol. Plant Pathol. 65, 11–29.

Gargouri, S., Hajlaoui, M.R., Guermech, A., Marrakchi, M., 2001. Identicifation des espèces fongiques associées à la pourriture du pied du ble et etude de leur répartition selon les étages bioclimatques en Tunisie. EPPO Bull. 31, 499–503.

Geisen, R., 1996. Multiplex polymerase chain reaction for the detection of potential aflatoxin and sterigmatocystin producing fungi. Syst. Appl. Microbiol. 19, 388–392.

Geisen, R., Mulfinger, S., Niessen, L., 1998. Detection of *Aspergillus flavus* in wheat by PCR. J. Food Mycol. 1, 211–218.

Gonzalez-Jaen, M.T., Mirete, S., Patino, B., Lopez-Errasquin, E., Covadonga, V., 2004. Genetic markers for the analysis of variability and for production of specific diagnostic sequences in fumonisin-producing strains of *Fusarium verticillioides*. Europ. J. Plant Pathol. 110, 525–532.

Gravesen, S., Frisvad, J.C., Samson, R.A., 1994. Microfungi. Munksgaard, Copenhagen. 168p.

Grimm, C., Geisen, R., 1998. A PCR-ELISA for the detection of potential fumonisin producing *Fusarium* species. Lett. Appl. Microbiol. 26, 456–462.

Grove, J.F., 1993. Macrocyclic trichothecenes. Nat. Prod. Rep. 10, 429–448.

Haugland, R.A., Vesper, S.J., 2000. Method for identifying and quantifying specific fungi and bacteria. US Patent and Trademark Office, Patent #6,387,652, May 14, 2002, 29 pp.

Hendricks, K., 1999. Fumonisins and neural tube defects in south Texas. Epidemiology 10, 198–200.

Hohn, T.M., McCormick, S.P., Desjardin, A.E., 1993. Evidence of a gene cluster involving trichothecene-pathway biosynthetic genes in *Fusarium sporotrichioides*. Curr. Genet. 24, 291–295.

IARC (International Agency for Research on Cancer), 1993. Toxins derived from *Fusarium* moniliforme: Fumonisins B_1 and B_2 and fusarin C. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans: Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromaotic Amines and Mycotoxins, vol. 56. International Agency for Research on Cancer, Lyon, France, pp. 445–466.

Jarvis, B.B., Mokhtari, R.N., Schenkel, E.P., Barros, C.S., Matzenbacher, N.I., 1991. Trichothecene mycotoxins from Brazilian *Baccharis* spp. Phytochemistry (Oxford) 30, 789–798.

Kampelmacher, E.H., 1973. Since Eve ate apples. Can. J. Public Health 64, 231–237.

Kimura, M., Tokai, T., O′Donnell, K., Ward, T.J., Fujimura, M., Hamamoto, H., Shibata, T., Yamaguchi, I., 2003. The trichothecene biosynthesis gene cluster of *Fusarium graminearum* F15 contains a limited number of essential pathway genes and expressed non-essential genes. FEBS Lett. 27, 105–110.

Klemsdal, S.S., Elen, O., 2006. Development of a highly sensitive nested-PCR method using a single closed tube for detection of *Fusarium* culmorum in cereal samples. Lett. Appl. Microbiol. 42, 544–548.

Knoll, A., Niessen, L., Vogel, R.F., 2000. Application of a PCR protocol for the diagnosis of trichothecene producing *Fusarium* species in deoxynivalenol contaminated wheat. Mycotoxin Res. 16A, 240–243.

Knoll, A., Vogel, R.F., Niessen, L., 2002. Identification of *Fusarium* graminearum in cereal samples by DNA Detection Test Strips™. Lett. Appl. Microbiol. 34, 144–148.

Koster, B., Scott, J., Wong, B., Malloch, D., Straus, N., 2003. A geographically diverse set of isoltates indicates two phylogenetic lineages within *Stachybotrys chartarum*. Can. J. Bot. 81, 633–643.

Kristensen, R., Berdal, K.G., Holst-Jensen, A., 2006a. Simultaneous detection and identification of richothecene- and moniliformin-producing *Fusarium* species based on multiplex SNP analysis. J. Appl. Microbiol. 102, 1071–1081.

Kristensen, R., Gauthier, G., Berdal, K.G., Hamels, S., Remacle, J., Holst-Jensen, A., 2006b. DNA microarray to detect and identify trichothecene- and moniliformin-producing *Fusarium* species. J. Appl. Microbiol. 102, 1060–1070.

Kulik, T., Fordonski, G., Pszczolkowska, A., Plodzien, K., Lapinski, M., 2004. Development of PCR assay based on ITS2 rDNA polymorphism for the detection and differentiation of *Fusarium sporotrichioides*. FEMS Microbiol. Lett. 239, 181–186.

Lee, T., Oh, D.W., Kim, H.S., Lee, J., Kim, Y.H., Yun, S.H., Lee, Y.W., 2001. Identification of deoxynivelenol- and nivalenol-producing chemotypes of *Gibberella zeae* by using PCR. Appl. Environ. Microbiol. 67, 2966–2972.

Leisova, L., Kucera, L., Chrpova, J., Sykorova, S., Sip, V., Ovesna, J., 2006. Quantification of *Fusarium* culmorum in wheat and barley tissues using real-time PCR in comparison with DON content. J. Phytopathol. 154, 603–611.

Leslie, J.F., 1995. *Gibberella fujikuroi*: available populations and variable traits. Can. J. Bot. 73 (Suppl. 1), S281–S291.

Lysoe, E., Klemsdal, S.S., Bone, K.R., Frandsen, R.J.N., Johansen, T., Thrane, U., Giese, H., 2006. The PKS4 gene of *Fusarium graminearum* is essential for zearalenone production. Appl. Environ. Microbiol. 72, 3924–3932.

Marek, P., Thirunavukkarasu, A., Kumar, V., 2003. Detection of *Penicillium expansum* by polymerase chain reaction. Int. J. Food Microbiol. 89, 139–144.

Mayer, Z., Bagnara, A., Färber, P., Geisen, R., 2003. Quantification of the copy number of nor-1, a gene of the aflatoxin biosynthetic pathway by real-time PCR, and its correlation to the cfu of *Aspergillus flavus* in foods. Int. J. Food Microbiol. 82, 143–151.

Merrill Jr., A.H., Sullards, M.C., Wang, E., Voss, K.A., Riley, R.T., 2001. Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. Environ. Health Perspect. 109 (suppl. 2), 283–289.

Mirocha, C.J., Pathre, S.V, Behrens, J., Schauerhamer, B., 1978. Uterotropic activity of cis and trans isomers of zearalenone and zearalenol. Appl. Environ. Microbiol. 35, 986–987.

Mishra, P.K., Fox, R.T.V., Culhan, A., 2003. Development of a PCR-based assay for rapid and reliable identification of pathogenic fusaria. FEMS Microbiol. Lett. 218, 329–332.

Möller, E.M., Chelkowski, J., Geiger, H.H., 1999. Species-specific PCR assays for the fungal pathogens *Fusarium moniliforme* and *Fusarium subglutinans* and their application to diagnose maize ear rot disease. J. Phytopathol. (Berlin) 147, 497–508.

Mulé, G., Susca, A., Stea, G., Moretti, A., 2004. A species-specific PCR assay based on the calmodulin partial gene for identification of *Fusarium verticillioides, F. proliferatum* and *F. subglutinans*. Europ. J. Plant Pathol. 110, 495–502.

Mulé, G., Susca, A., Logrieco, A., Stea, G., Visconti, A., 2006. Development of a quantitative real-time PCR assay for the detection of *Aspergillus carbonarius* in grapes. Int. J. Food Microbiol. 111, 28–34.

Murillo, I., Cavallarin, L., San-Segundo, B., 1998. The development of a rapid PCR assay for detection of *Fusarium moniliforme*. Europ. J. Plant Pathol. 104, 301–311.

Muture, B.N., Ogana, G., 2005. Aflatoxin levels in maize and maize products during the 2004 food poisoning outbreak in eastern province of Kenya. East Afr. Med. J. 82, 275–279.

Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W., Joyce, D., 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Phys. Mol. Plant Pathol. 53, 17–37.

Niessen, L., 2007. Current trends in molecular diagnosis of ochratoxin A producing fungi. In: Rai, M. (Ed.), Mycotechnology—Present Status and Future Prospects. I.K. International Publishing House, New Delhi. 320 pp.

Niessen, M.L., Vogel, R.F., 1997. Specific identification of *Fusarium graminearum* by PCR with gaoA targeted primers. Syst. Appl. Microbiol. 20, 111–123.

Niessen, M.L., Vogel, R.F., 1998. Group specific PCR-detection of potential trichothecene-producing *Fusarium*-species in pure cultures and cereal samples. Syst. Appl. Microbiol. 21, 618–631.

Niessen, L., Klusmann, J., Vogel, R.F., 1998. Quantitative estimation of *Fusarium graminearum* DNA using a solid phase PCR assay (DIAPOPS). J. Food Mycol. 1, 73–84.

Niessen, L., Schmidt, H., Vogel, R.F., 2004. The use of tri5 gene-sequences for PCR detection and taxonomy of trichothecene-producing species in the *Fusarium* section *Sporotrichiella*. Int. J. Food Microbiol. 95, 305–319.

Obradovic, D., Bagi, F., Kevresan, S., Petrovic, T., Balaz, F., 2001. A simplified method of DNA extraction for identification of *Fusarium graminearum* group 2 isolates by PCR. Acta Phytopathol. Entomol. Hung. 36 (3–4), 243–249.

Paterson, R.R.M., 2004. The isoepoxydon dehydrogenase gene of patulin biosynthesis in cultures and secondary metabolites as candidate PCR inhibitors. Mycol. Res. 108, 1431–1437.

Paterson, R.R.M., 2006. Primers from the isoepoxydon dehydrogenase gene of the patulin biosynthetic pathway to indicate critical control points for patulin contamination of apples. Food Control 17, 741–744.

Paterson, R.R.M., Archer, S., Kozakiewicz, Z., Lea, A., Locke, T., O´Grady, E., 2000. A gene probe for the patulin metabolic pathway with potential for use in patulin and novel disease control. Biocontrol Sci. Technol. 10, 509–512.

Patino, B., Mirete, S., Gonzalez-Jaen, M.T., Mule, G., Rodriguez, T., Velazquez, C., 2004. PCR detection assay of the fumonisin producing *Fusarium verticillioides* strains. J. Food Prot. 67, 1278–1283.

Pedersen, L.H., Skouboe, P., Boysen, M., Soule, J., Rossen, L., 1997. Detection of *Penicillium* species in complex food samples using the polymerase chain reaction. Int. J. Food Microbiol. 35, 169–177.

Peltola, S., Niessen, L., Nielsen, K.F., Jarvis, B.B., Andersen, B., Salkinoja-Salonen, M., Möller, E.M., 2002. Toxigenic diversity of two different RAPD groups of *Stachybotrys chartarum* isolates analyzed by potential for trichothecene production and for boar sperm cell motility inhibition. Can. J. Microbiol. 48, 1017–1029.

Peraica, M., Radic, B., Lucic, A., Pavlovic, M., 1999. Toxic effects of mycotoxin in humans. Bull. WHO 77, 754–756.

Pitt, J.I., 2000. Toxigenic fungi and mycotoxins. Brit. Med. Bull. 56, 184–192.

Reischer, G.H., Lemmens, M., Farnleitner, A., Adler, A., Mach, R.L., 2004. Quantification of *Fusarium graminearum* in infected wheat by species specific real-time PCR applying a TaqMan probe. J. Microbiol. Methods 59, 141–146.

Rheeder, J.P., Marasas, W.F., Vismer, H.F., 2002. Production of fumonisin analogs by *Fusarium* species. Appl. Environ. Microbiol. 68, 2102–2105.

Rosso, L., Maier, M.S., Bertoni, M.D., 2000. Trichothecenes production by the hypocrealean epibiont of *Baccharis coridifolia*. Plant Biol. (Stuttg.) 2, 684–686.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Sharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Ehrlich, H.A., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239, 487–491.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C. (eds.), 2004. Introduction to Food- and Airborne Fungi. Centralbrueau voor Schimmelcultures, Utrecht, Netherlands, pp. 1–282.

Sanchez-Rangel, D., SanJuan-Badillo, A., Plasencia, J., 2005. Fumonsin production by *Fusarium verticillioides* trains isolated from maize in Mexico and development of a polymerase chain reaction to detect potential toxigenic strains in grains. Agric. Food Chem. 53, 8565–8571.

Sarlin, T., Yli-Mattila, T., Jestoi, M., Rizzo, A., Paavanen-Huhtala, S., Haikara, A., 2006. Real-time PCR for quantification of toxigenic *Fusarium* species in barley and malt. Europ. J. Plant Pathol. 114, 371–380.

Schilling, A.G., Moeller, E.M., Geiger, H.H., 1996. Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum, F. graminearum* and *F. avenaceum*. Phytopathology 86, 515–522.

Schmidt, H., Bannier, M., Vogel, R.F., Niessen, L., 2004. Detection and quantification of *Aspergillus ochraceus* in green coffee by PCR. Lett. Appl. Microbiol. 38, 464–469.

Schnerr, H., Niessen, L., Vogel, R.F., 2001. Real time detection of the tri5 gene in *Fusarium* species by LigthCycler-PCR using SYBR green 1 for continuous fluorescence monitoring. Int. J. Food Microbiol. 71, 53–61.

Schnerr, H., Vogel, R.F., Niessen, L., 2002. Correlation between DNA of trichothecene-producing *Fusarium* species and deoxynivalenol concentrations in wheat samples. Lett. Appl. Microbiol. 35, 121–125.

Sekiguchi, J., Gaucher, G.M., 1979. Isoepoxydon, a new metabolite of the patulin pathway in *Penicillium urticae*. Biochem. J. 182, 445–453.

Shapira, R., Paster, N., Eyal, O., Menasherov, M., Mett, A., Salomon, R., 1996. Detection of aflatoxingenic molds in grains by PCR. Appl. Environ. Microbiol. 62, 3270–3273.

Strausbaugh, C.A., Overturf, K., Koehn, A.C., 2005. Pathogenicity and real-time PCR detection of *Fusarium* spp. in wheat and barley roots. Can. J. Plant Pathol. 27, 430–438.

Sugita, T., Kamimura, K., Uchida, K., Yamaguchi, H., Nagai, A., 2004. PCR identification system for the genus *Aspergillus* and three major pathogenic species: *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger*. Med. Mycol. 42, 433–437.

Sydenham, E.W., Shephard, G.S., Thiel, P.G., Marasas, W.F.O., Stockenstrom, S., 1991. Fumonisin contamination of commercial corn-based human foodstuffs. J. Agric. Food Chem. 39, 2014–2018.

Tan, M.K., Niessen, L., 2003. Analysis of rDNA ITS sequences to determine genetic relationships among, and provide a basis for simplified diagnosis of *Fusarium* species causing crown rot and head blight of cereals. Mycol. Res. 107, 811–821.

Tang, C.M., Holden, D.W., Aufauvre-Brown, A., Cohen, J., 1993. The detection of *Aspergillus* spp. by the polymerase chain reaction and its evaluation in bronchoalveolar lavage fluid. Am. Rev. Respir. Dis. 148, 1313–1317.

Torp, M., Nirenberg, H.I., 2004. *Fusarium langsethiae* sp. nov. on cereals in Europe. Int. J. Food Microbiol. 95, 247–256.

Trapp, S.C., Hohn, T.M., McCormick, S., Jarvis, B.B., 1998. Characterization of the gene cluster for biosynthesis of macrocyclic trichothecenes in *Myrothecium roridum*. Mol. Gen. Genet. 257, 421–432.

Turner, W.B., Aldridge, D.C., 1983. Fungal Metabolites II. Academic Press, London. 631 pp.

Waalwijk, C., van der Lee, T., de Vries, I., Hesselink, T., Arts, J., Kema, G.H.J., 2004a. Synteny in toxigenic *Fusarium* species: the fumonisin gene cluster and the mating type region as examples. Europ. J. Plant Pathol. 110, 533–544.

Waalwijk, C., van der Heide, R., de Vries, T., van der Lee, T., Schoen, C., Costrel-de Corainvill, G., Häuser-Hahn, I., Kastelein, P., Köhl, J., Lonnet, P., Demarquet, T., Kema, G.H.J., 2004b. Quantitative detection of *Fusarium* species in wheat using TaqMan. Europ. J. Plant Pathol. 110, 481–494.

Williams, K.J., Dennis, J.I, Smyl, C., Wallwork, H., 2002. The application of species-specific assays based on the polymerase chain reaction to analyse *Fusarium* crown rot of durum wheat. Australas. Plant Pathol. 31, 119–127.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18, 6531–6536.

Yoder, W.T., Christianson, L.M., 1998. Species-specific primers resolve members of *Fusarium* section *Fusarium*: Taxonomic status of the edible "Quorn" fungus reevaluated. Fung. Gen. Biol. 23, 68–80.

Zheng, Q., Ploetz, R., 2002. Genetic diversity in the mango malformation pathogen and development of a PCR assay. Plant Pathol. 51, 208–212.