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Verification of the effectiveness of SCAR (sequence characterized amplified region) primers for the identification of Polish strains of *Fusarium culmorum* and their potential ability to produce B-trichothecenes and zearalenone

Anna Baturo-Ciesniewska [a,*], Michalina Suchorzynska [b]

[a] University of Technology and Life Sciences, Faculty of Agriculture and Biotechnology, Department of Phytopathology and Molecular Mycology, Kordeckiego St. 20, 85-225 Bydgoszcz, Poland
[b] Institute of Agricultural and Food Biotechnology, Department of Microbiology, Rakowiecka St. 36, 02-532 Warszawa, Poland

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ABSTRACT

Rapid and sensitive methods to detect *Fusariumculmorum* and trichothecene and zearalenone producing strains in food and feed are valuable in predicting potential contamination. In this study the effectiveness of primers, recommended in the literature, for species identification of *F. culmorum* and basic genes encoding for mycotoxin production was tested. A total of 68 isolates of *F. culmorum* were collected from cereals and potato between 2005 and 2008 from different Polish provinces. It was shown that from among the four primer pairs enabling the identification of *F. culmorum*, and therefore also to establish its presence in the material, only primers Fc01F/Fc01R seem to be fully effective in the case of Polish strains. Determination of material contamination by *F. culmorum*, however, is only a first step in determining food safety. It is also extremely important to identify genes encoding the potential ability to produce mycotoxins. It was shown that three pairs of primers (tox5-1/tox5-2, HATriF/HATriR and Tri5F/Tri5R) enable a fully effective identification of the presence of the *Tri5* gene responsible for producing trichothecenes. Determination of the DON-chemotype, and thus identification of the strains of *F. culmorum* potentially producing deoxynivalenol, is enabled equally by MinusTri7F/MinusTri7F, Tri7F/Tri7DON and Tri13F/Tri13DONR. However, a determination of the NIV-chemotype, and thus identification of the strains potentially producing nivalenol, is enabled by Tri7F/Tri7R, Tri7F/Tri7NIV and Tri13NIVF/Tri13R. The potential ability of isolates to produce ZEA can be determined to the same degree in assay with PKS4-PS.1/PKS4-PS.2 and F1/R1.

1. Introduction

Diagnosis of fungi occurring on food and feed plants and development of effective methods for their identification are extremely important. *Fusarium* species are widespread in the environment and may be serious threat to plant and human health. In Poland, as elsewhere, attention is given to species occurring mainly on cereals such as *F. culmorum* (W.G. Smith) Sacc., *F. graminearum* Schwabe, *F. avenaceum* (Corda ex Fries) Sacc. and *F. poae* (Peck) Wollenw. (Chełkowski, 1998; Golinski et al., 2002; Nicholson et al., 2004). Less attention is paid to the fact that *Fusarium* spp. also infect potatoes, where in addition to *F. sambucinum* (Fuckel), *F. culmorum* also occurs (Lenc et al., 2008; Peters et al., 2008). Plant infection with *Fusarium* spp. can lead to an accumulation of the mycotoxins produced by them (Bottalico and Perrone, 2002; Nielsen et al., 2009; Perkowski et al., 2003). Mycotoxins have been found to cause a variety of toxic effects on humans and livestock (Desjardins, 2006; Pestka, 2007). *Fusarium culmorum*, one of the common pathogens of many important plants is able to produce B-trichothecenes and zearalenone (Chandler et al., 2003; Lysøe et al., 2006; Meng et al., 2010; Tomczak et al., 2002).

Traditional methods to identify *Fusarium* species using a microscope and mycological keys are relatively simple, but sometimes are inefficient, time-consuming and require experienced operators. The identification of individual species of *Fusarium* is difficult because of the high variability of morphological features, which may depend on external conditions, and differing classification systems (Leslie et al., 2001; Leslie and Summerell, 2006; Yoder and Christianson, 1998). Molecular techniques are more sensitive, faster, and do not require a lot of experience in plant pathology (McCartney et al., 2003). For over 10 years numerous PCR-based methods for diagnosis and quantification of *Fusarium* species, with a determination of their potential ability to produce mycotoxins such as deoxynivalenol and nivalenol, have been intensively described (Chandler et al., 2003; Jennings et al., 2004; Niessen, 2007). Identification of the presence of the *Tri* cluster genes, such as *Tri5*, *Tri7*, *Tri13* and *Tri3* in the genome makes it possible to determine the potential ability of *Fusarium* species to produce trichothecenes and to distinguish among them isolates that are

* Corresponding author. Tel.: +48 52 374 93 42; fax: +48 52 374 93 27.
E-mail address: baturo-a@utp.edu.pl (A. Baturo-Ciesniewska).

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potentially able to synthesize their specific type (chemotypes DON, including 3A-DON, 15A-DON, and NIV) (Chandler et al., 2003; Edwards et al., 2001; Jennings et al., 2004; Lee et al., 2001; Nicholson et al., 2004).

Although most attention is paid to the trichothecene mycotoxins, a number of other mycotoxins, including zearalenone, are of significance in diseases of cereals and in food contamination. Detection and quantification of zearalenone-producing *Fusarium* strains has not been reported so far (Meng et al., 2010). Their detection can be based on an analysis of *PKS* genes, especially *PKS4* that has been reported to be essential in the production of zearalenone (polyketide mycotoxin) (Lysøe et al., 2006). Identification of the presence of *F. culmorum* and the genes responsible for the potential ability to produce zearalenone and trichothecenes is therefore very important, because it may enable the prevention of contamination of food or feed by this mycotoxin.

According to data in the literature, SCAR (sequence characterized amplified region) markers do not always guarantee the real characteristics of isolates which, despite belonging to the same species, may differ on a molecular level. Therefore, in this study, the effectiveness of 17 primer pairs for species identification of Polish strains of *F. culmorum* and basic genes encoding mycotoxin production was tested.

2. Material and methods

2.1. Collection and identification of F. culmorum isolates and preparation of single spore cultures

Sixty eight isolates of *F. culmorum* were collected from winter and spring wheat, spring barley, triticale, winter rye, oats and potato between 2005 and 2008 from different Polish provinces (Table 1). Sampling was conducted from stem bases, roots and harvested grain of cereals, and from potato tubers with disease symptoms.

To isolate the fungus the plant material was surface-disinfected for 2 min in 1% sodium hypochlorite, rinsed three times in sterile water, dried on sterile blotting paper and placed onto potato dextrose agar (PDA, Difco, USA). After 7 days of incubation in the dark at 23 °C, cultures were placed first into tubes with a PDA medium, next onto Petri dishes with a PDA or SNA medium and identified based on conidial morphology (Leslie and Summerell, 2006).

Two weeks later, single spore cultures were prepared. All isolates were placed on Petri dishes with an SNA medium and incubated at 23 °C for 10 days. Next, a small amount of sporulating mycelium was smeared onto 2% water agar. Sixteen hours later, single germinated conidia, identified using a microscope, were transferred, using a

Table 1
Details of amplification conditions including sequences of primers, their authors and the literature source, annealing temperature, extension time and cycle number.

Symbol of analysis	Primers	Sequence	Assay	PCR conditions	Product size (bp)		Source
Fc N	Fc01F Fc01R	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	*F. culmorum*	62 °C Touchdown[a]	570		Nicholson et al., 1998
Fc S	$OPT18F_{470}$ $OPT18R_{470}$	GATGCCAGACCAAGACGAAG GATGCCAGACGCACTAAGAT	*F. culmorum*	55 °C, 30 s, 30 cycles	472		Schilling et al., 1996
Fc L	FcF FcR	CAAAAGCTTCCCGAGTGTGTC GGCGAAGGTTCAAGGATGAC	*F. culmorum*	60 °C, 30 s, 40 cycles	700		Lees, 1995; Doohan et al., 1998
Fc J	Fcu-F Fgc-R	GACTATCATTATGCTTGCGAGAG CTCTCATATACCCTCCG	*F. culmorum*	54 °C, 30 s, 20 cycles	200		Jurado et al., 2005
Tox5	Tox5-1 Tox5-2	GCTGCTCATCACTTTGCTCAG CTGATCTGGTCACGCTCATC	*Tri5* gene	62 °C, 30 s, 30 cycles	658		Niessen and Vogel, 1998
Ha Tri	HA TriF HA TriR	CAGATGGAGAACTGGATGGT GCACAAGTGCCACGTGAC	*Tri5* gene	62 °C, 15 s, 35 cycles	260		Edwards et al., 2001
Tri5	Tri5F Tri5R	AGCGACTACAGGCTTCCCTC AAACCATCCAGTTCTCCATCTG	*Tri5* gene	60 °C, 30 s, 30 cycles	545		Nicholson et al., 2004
PKS 4-1	PKS4-PS.1 PKS4-PS.2	GTGGGCTTCGCTAGACCGTGAGTT ATGCCCTGATGAAGAGTTTGA	PKS4 (zearalenone)	59 °C, 30 s, 35 cycles[b]	400		Lysøe et al., 2006
PKS 4-2	F1 R1	CGTCTTCGAGAAGATGACAT TGTTCTGCAAGCACTCCGA	PKS4 (zearalenone)	60 °C, 30 s, 35 cycles	~280		Meng et al., 2010
Fg16N	Fg16NF Fg16NR	ACAGATGACAAGATTCAGGCACA TTCTTTGACATCTGTTCAACCCA	*F. graminearum*	62 °C Touchdown	280		Nicholson et al., 1998
					Chemotype DON	Chemotype NIV	
Minus Tri7	MinusTri7F MinusTri7R	TGGATGAATGACTTGAGTTGACA AAAGCCTTCATTCACAGCC	Minus Tri7	58 °C, 30 s, 35 cycles	483	–	Chandler et al., 2003
Tri7 DON	Tri7F Tri7DON	TGCGTGGCAATATCTTCTTCTA GTGCTAATATTGTGCTAATATTGTGC	Tri7DON	60 °C, 30 s, 35 cycles	381–445	–	Chandler et al., 2003
Tri13 DON	Tri13F Tri13DONR	CATCATGAGACTTGTKCRAGTTTGGG[c] GCTAGATCGATTGTTGCATTGAG	Tri13DON	58 °C, 45 s, 35 cycles	282	–	Chandler et al., 2003
Tri7gener	Tri7F Tri7R	TGCGTGGCAATATCTTCTTCTA TGTGGAAGCCGCAGA	GenericTri7	60 °C, 30 s, 35 cycles	–[d]	436	Chandler et al., 2003
Tri7 NIV	Tri7F Tri7NIV	TGCGTGGCAATATCTTCTTCTA GGTTCAAGTAACGTTCGACAATAG	Tri7 NIV	60 °C, 30 s, 35 cycles	–	465	Chandler et al., 2003
Tri13 NIV	Tri13NIVF Tri13R	CCAAATCCGAAAACCGCA TTGAAAGCTCCAATGTCGTG	Tri13 NIV	58 °C, 45 s, 35 cycles	–	312	Chandler et al., 2003
Tri303	Tri303F Tri303R	GATGGCCGCAAGTGGA GCCGGACTGCCCTATTG	3A-DON	55 °C, 30 s, 30 cycles[b]	586	–	Jennings et al., 2004
Tri315	Tri315F Tri315R	CTCGCTGAAGTTGGACGTAA GTCTATGCTCTCAACGGACAAC	15A-DON	58 °C, 45 s, 30 cycles[b]	864	–	Jennings et al., 2004

[a] Explained in Material and methods.
[b] Author's PCR conditions.
[c] K = G + T, R = A + G.
[d] For *F. culmorum* isolates.